CYTOGENETICS AND SEED-SET OF AUTOTETRAPLOID RYE, SECALE CEREALE L.

M. Gul Hossain

A Thesis Submitted for the Degree of PhD
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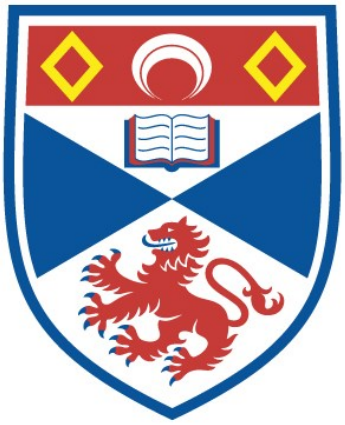

1975

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Ph.D. Thesis

by

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ABSTRACT

Twenty years after chromosome doubling an unselected population of tetraploid rye appeared to have reached an "equilibrium state" in chromosome pairing behaviour. Cytogenetically the population was highly heterogeneous. Compared to early C-generations, meiotic behaviour in the population improved by an increase in quadrivalent frequency, mainly at the expense of trivalents and univalents.

Quadrivalent frequency, however, failed to correlate with other meiotic features; instead bivalent frequency had significant positive correlations with the features of meiotic regularity including chiasma frequency. Furthermore, the average quadrivalent frequency in the population was considerably less than that of inbred lines (Hazarika & Rees, 1967). These facts led to the conclusion that disomic pairing dominated the chromosome behaviour in this random mating population. No meiotic features nor morphological characters were correlated with seed-set in the population.

A simultaneous selection for seed-set and regular tetrads was effective in increasing the bivalent frequency and reducing the frequencies of quadrivalents and aneuploids.

A comparative study of three populations ("high", "low" and unselected) indicated the variable relationship between chiasma frequency and the frequencies of quadrivalents and bivalents. This and the higher frequency of quadrivalents in inbred materials was explained on the basis of "free" and "restricted" pairing of the four homologous chromosomes of an autotetraploid. It was concluded that the pairing pattern in inbred materials is predominantly tetrasomic whereas in outbred materials this may vary from tetrasomic to disomic depending on the chromosomal differentiation within the homologous sets.

In the unselected population the lack of correlation of seed-set with meiotic features and morphological characters proved to be due to a supplementary interaction between the cytological and the so-called physiological factors in determining the fertility of a plant. It was demonstrated that the genetical control of the cytological factors is independent from that of the so-called physiological factors. Once the interaction due to the latter was reduced by selection pressure, the effects of the cytological factors on seed-set became evident. But depending on whether the pairing pattern is predominantly tetrasomic or disomic, seed-set is correlated with quadrivalent or bivalent frequency.

Quadrivalents were found to be sensitive to environmental changes whereas bivalents remained relatively stable. Furthermore, quadrivalent formations are restricted with 2/3 of the chromosomes. It is, therefore, concluded that the only way of ensuring meiotic stability in an autotetraploid is to induce disomic pairing. The possible ways of achieving this are outlined.

Several chromosomal aberrations detected during the investigation are illustrated and discussed.

DECLARATION

I declare that the thesis has been composed by me, that the work of which it is a record has been done by myself, and that it has not been accepted in any previous application for a higher degree.

Name _____

(M. GUL HOSSAIN)

Date 18th April, 1975

CYTOGENETICS AND SEED-SET OF AUTOTETRAPLOID RYE (*Secale cereale* L.)

being a thesis submitted by

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towards the degree of

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Department of Botany

University of St. Andrews

March, 1975

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I certify that M. Gul Hossain has spent over nine terms of research work under the direction of Dr. K. Moore and myself and that he has fulfilled the conditions of Ordinance No. 12 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of DOCTOR OF PHILOSOPHY.

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(Prof. J. A. Macdonald)

Department of Botany

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SUMMARY

1. Meiotic studies in a population of 'Fourex' spring rye, after about 20 years of chromosome doubling, showed an increase in quadrivalent frequency mainly at the expense of trivalents and univalents. Cytogenetically the population was heterogeneous as revealed by highly significant differences between plants with respect to chiasma frequency, univalent frequency and the proportion of regular metaphase-I cells and regular tetrads. The plants within the population, however, did not differ with regard to the frequencies of multivalents and bivalents nor was the cell-variance for chiasmata correlated with other meiotic features. This was explained as an "equilibrium state" of the population with regard to its chromosome pairing behaviour.

In contrast to inbred materials, quadrivalent frequency failed to show any significant correlation with chiasma frequency; instead bivalent frequency had a positive and significant correlation with chiasma frequency. This property and a considerable reduction in the number of chromosomes involved in multivalent formations, as compared to inbred materials, indicated that a high degree of "pairing restriction" in favour of bivalent formation dominated the pairing pattern in this random mating population. It is further indicated that in this population bivalent formation is easily and more efficiently accomplished than multivalent formation.

Seed-set was not correlated with any of the cytological features or the morphological characters. The lack of correlations was proposed to be due to an interaction between the cytological and the so-called physiological factors in determining the fertility of a plant.

2. A simultaneous selection for seed-set and meiotic behaviour over four generations was effective in reducing the frequencies of aneuploids and increasing bivalent frequency significantly, with a corresponding decrease in quadrivalents.

A comparative study of three populations, namely "high", "low" and "unselected", indicated the variable relationship between chiasma frequency on one hand and the frequencies of quadrivalents and bivalents on the other. The "high" population showed a positive and significant correlation between chiasma frequency and quadrivalents whereas in the "low" and the unselected populations chiasma frequency was strongly correlated with bivalents. This variation in the relationship and the differences in pairing configuraions were explained on the basis of "free" and "restricted" pairing pattern within the four homologous chromosomes of an autotetraploid.

As regards seed-set, its dependence on meiotic behaviour and plant vigour varied for the three populations. It was demonstrated that in the advanced unselected population the

cytological and the so-called physiological factors supplement each other in determining seed-set and this relationship can be easily broken by selection pressure. This led to the conclusion that the genetic basis of cytological factors is independent from that of the so-called physiological factors. This signifies the need for selection for both sets of factors for fertility-improvement in an autotetraploid population.

3. In a study of the effects of external environmental factors on chromosome association in autotetraploid plants, it was demonstrated that one can identify genotypes with meiotic stability from genotypes which are less stable. It was also shown that multivalents are highly sensitive to environmental changes whereas bivalents in general are relatively stable configurations. The underlying mechanisms which provide bivalents with a better stability are discussed.

4. The following chromosomal abnormalities were studied during the course of the investigation

   (a) Aneuploidy: There were 16%, 29% and 23% aneuploids in the "high", the "low" and the unselected populations respectively. The reduced level of aneuploid frequency in the "high" population seemed to be associated with higher frequency

of bivalents. While the aneuploids were, in general, inferior to euploids both in fertility and vigour, there were no significant differences between various aneuploid groups (i.e. hypo- and hyperaneuploids) for seed-set and morphological characters nor was there any significant difference between aneuploids of the three populations.

(b) Translocation Heterozygotes: Eight plants heterozygous for spontaneous interchanges were identified. Some of these plants did not show the typical octavalent formation at metaphase-I and as a result an indirect method was adopted for the identification of interchange heterozygotes.

(c) Anaphase Bridges with or without Fragments as a result of Errors in Crossing-over.

(d) Paracentric Inversion.

(e) Centric Fragments.

(f) Neo-Centric Activity.

(g) Polyad Formation.

(h) Abnormality in Spindle Mechanism and Cell-Wall Formation.

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SECTION THREE

SECTION FOUR

GENERAL INTRODUCTION

The phenotypic superiority of natural polyploids over the related diploid species has stimulated plant breeders' interests in the production of artificial polyploids. While the 'gigas' nature of polyploids provides the basis for higher yields, chromosome doubling also allows an extension of the breeding unit by overcoming the sterility barrier between species, as well as by weakening the gametophytic incompatibility that exists at the diploid level. Thus from the breeding point of view, polyploidy seems to have great prospects and the discovery of colchicine (Dustin, Havas & Lits, 1937) and its successful application in chromosome doubling (Blakeslee & Avery, 1937; Eigsti, 1938; Nebel & Ruttle, 1938) promoted widespread experimental works on polyploidy.

Although many of the phenotypic changes in induced polyploids are of potential value to plant breeders, the major drawback, however, has been the reduced fertility, especially in autopolyploids. Levan (1945) made a survey of various artificial polyploids and put forward three basic criteria for successful polyploidy breeding. These are (a) allogamous plants with (b) low chromosome number, grown for (c) vegetative yields. The examples that are well known include sugar beet (*Beta vulgaris*), watermelon (*Colocynthus citrullus*), root turnip (*Brassica campestris*), red and alsike

clover (*Trifolium spp.*) and forage kale (*Brassica oleracea*). But the problem of reduced fertility, with grain crops in particular, still remains the major limiting factor. Consequently induced polyploids have been of greatest interest in cytological and genetical studies and their potential value in crop improvement continues to be explored.

Of the three requirements for successful breeding at polyploid level (Levan, l.c.), rye fulfills the first two i.e. allogamous breeding system and low number of chromosomes. Added to this concerted efforts have been made to improve the fertility in polyploid forms ever since the first tetraploid was obtained (Dorsey, 1936; Lamm, 1936; Muntzing, 1936). Also the incompatibility system in rye, unlike some other artificial polyploids, for instance white clover (Brewbaker, 1955), does not break down at the tetraploid level and this helps to maintain the outbreeding system and heterosis. Furthermore rye, being an excellent material for cytogenetical studies, seems to have received a privileged attention. These special circumstances undoubtedly facilitated efforts to improve the seed-yield. As a result several varieties of tetraploid rye have been developed and proved successful in agriculture (Muntzing, 1951 & 1954; Hagberg & Ellerström, 1959; Wexelsen, 1961; Aastveit, 1963 & 1968).

The tetraploid strains, although not superior in yield to the diploid varieties, possess other advantageous sub-

characters such as higher grain weight (Muntzing, 1951; Plarre, 1954), superior baking quality (Muntzing, 1951; Hintzer & Miranda, 1954), reduced shattering of grains before harvest (Plarre, 1954), higher disease resistance (Wexelsen, et al., 1961), better sprouting ability (Muntzing, 1951), good straw-stiffness and better winter-survival (Wexelsen, et al,, 1961) as well as higher protein content (Noggle, 1947). With these improvements a general conclusion has been drawn that the real task of plant breeding only begins once the tetraploid has been created, because the newly synthesised tetraploids are "raw" polyploids where selection can lead to an improvement in their fertility (Muntzing, 1951; Allard, 1960,pp. 412; Ellerström & Sjodin, 1963; Williams, 1964 , pp. 452; Lewis, 1967; Dogget & Majisu, 1972).

However, seed-fertility in rye, measured in terms of percentage of seed-set, is considerably lower in tetraploid varieties compared to the diploids. Muntzing (1951) reported about 20% reduction in tetraploid populations. Morrison (1956) observed a reduction of at least 10%. Plarre (1954) found 71.3% seed-set in tetraploids against a corresponding value of 84.6% in diploids. Similar observations have been made by several other workers.

The reduced level of fertility is frequently exaggerated by the presence of aneuploids in the population and evidence from several works clearly shows that the average seed-set of

a population is inversely correlated with the frequency of aneuploids present in the population (Bremer & Bremer-Reinders, 1954; Hagberg & Ellerström, 1959; Wexelsen, et al., 1961; Aastveit, 1968). Conditions favouring higher seed-set in a tetraploid population appear to favour the development of aneuploid zygotes (see Hagberg & Ellerström, 1959; Rommel on barley, 1961; Ellerström & Sjodin, 1963; Moore, 1963). Such a situation implies that selection for seed-set could well be disadvantageous if care is not taken to avoid the increase in the number of aneuploids. While simple selection in "raw" populations can produce positive results, selection in an advanced population , where aneuploid frequency is one of the main limiting factors, would be far more difficult.

There are good reasons for believing that the chromosome behaviour in an autotetraploid is the major cause of reduced fertility and the occurrence of aneuploid individuals (Muntzing, 1936; Darlington, 1937; Myers & Hill, 1942). Tetraploid rye is no exception in this respect. On the other hand, physiological factors affecting gametic, zygotic and endospermic development are also known to be responsible for the lower level of fertility in autopolyploids (Stebbins, 1950; Håkansson & Ellerström, 1950; Moore, 1963). Morrison (1956) and Morrison & Rajhathy (1960) seem to attribute more emphasis to physiological and/or hereditary cause for the differences in fertility levels among autopolyploid populations. Other workers disagree as to the meiotic chromosome configurations

that affect fertility and suggest that multivalent associations in general are responsible for irregular chromosome disjunction and consequent low fertility (Darlington, 1937; Kostoff, 1939; Muntzing, 1951), while others showed that higher quadrivalent frequency is associated with higher fertility (Roseweir & Rees, 1962; Hazarika & Rees, 1967; Crowley & Rees, 1968). Still others are of the opinion that quadrivalent frequency is of minor importance in meiotic regularity and fertility (Myers & Hill, 1941; Myers, 1943, 1945 & 1947). McCollum (1958) observed the highest seed-set in the population of Dactylis which exhibited the lowest mean number of quadrivalents. Several other workers claim an increase in bivalent frequency with the increase in fertility (Plarre, 1954; Hilpert, 1957; Aastveit, 1968; Simonsen, 1973). Walther (1959) and Moore (1963), on the other hand, found no correlation between the degree of meiosis regularity and fertility; the former worker held the aneuploids in his sample to be responsible for the failure of the correlation while the latter worker, having excluded the aneuploids, believes that physiological factors disturb the relationship.

An examination of the literature indicates, therefore, that the relationship between meiotic regularity and seed-set may not be a simple one. One possible explanation for the wide range of disagreement among workers may be that both cytological and "other factors" are operative in determining fertility in most materials, the relative importance of one

over the other depending on the state of the experimental material with respect to its selective advancement following chromosome doubling. While "raw" and inbred populations are relatively unbalanced meiotically and hence genetically, an advanced population seems to attain a "threshold level" of stability and fertility beyond which any short term selection, natural or artificial, is hardly effective. At the same time it is conceivable that the effect of "other factors" may become more pronounced in advanced populations.

This necessitates a detailed investigation of both meiotic behaviour and fertility in advanced populations. Although many results of meiotic studies on tetraploid rye are available, it is significant that data from a sufficient number of individuals from any single population is surprisingly lacking. Muntzing (1951) represented each strain he studied by a single plant. Morrison (1956) compiled his meiosis data from only four plants. Bremer & Bremer-Reinders (1954) gave data for anaphase laggards and tetrad micronuclei from 9 and 13 plants respectively, but no data for metaphase analysis appear in their paper. On the other hand, detailed investigations by Roseweir & Rees (1962) and Hazarika & Rees (1967) were carried out in inbred lines which, as mentioned above, show more instability compared to normal outbred populations.

In the present investigation an attempt was made to study an advanced population of tetraploid spring rye in as much detail as possible. Fortunately the material used here has been studied in the past by Hilpert (1957) and Moore (1963) at different C-generations using large numbers of plants. While it is appreciated that the earlier investigations of the material were conducted in different climatic conditions (Sweden and California), the present investigation would nevertheless throw light on the changes, if any, that have occurred during a period of about 20 years.

The principal objectives of the investigation may be summarised as follows:

1. An assessment of the "threshold level" of stability and fertility in the population 20 years after chromosome doubling.

2. Effects of simultaneous selection for seed-set and meiotic regularity.

3. An analysis of the cytogenetical basis of seed-set.

4. Effects of some external environmental factors (Temperature and Nitrogen) on chromosome pairing behaviour in autotetraploid.

S E C T I O N O N E

THE UNSELECTED POPULATION

MATERIALS AND METHODS

The tetraploid spring rye used in the present investigation was originally obtained from the Swedish Seed Association, Svalöf, Sweden. The hybrid variety SV0201, obtained by crossing between Petkus and Od, was treated with colchicine in 1950 (see Hilpert, 1957). Later Hilpert (l.c.) and Moore (1963) made separate cytological investigations at different C-generations of the material. Dr. Moore brought a seed-sample of this tetraploid material to Britain in 1966 and started a selection programme (described in Section Two under Materials and Methods). In 1972 the present author carried out a comparison trial involving three groups of plants. Of these, two groups, "high" and "low" were materials selected for high and low seed-set respectively and the third group represented the unselected population of the material. The unselected population belonged to the same C-generation as the selected materials "high" and "low". The results obtained from this unselected population are reported in this section.

Cytological Methods

Young spikes were fixed in acetic-alcohol (1:3) for meiotic studies. As many plants as possible were fixed and the fixing continued from late-May to mid-July, 1972. This was done in order to avoid any sampling bias in fixing early flowering plants which could be different in chromosome constitution, vigour and so on from the late flowering ones. The fixed materials were temporarily stored in a refrigerator at 3°C to 5°C and later transferred to 70% alcohol and preserved in the refrigerator until they were cytologically examined.

The Squash Technique: In earlier stages of the work, slides were prepared according to the standard aceto carmine stain technique. Later Snow's Carmine (1963) was used and better preparations were obtained for the study of metaphase-I configurations. The author, however, observed that a simple modification of the iron-aceto-carmine technique gave as good preparations as with Snow's Carmine. The procedure is outlined below:

1. Dissect out the anther and smear in a drop of iron-aceto-carmine on a slide.

2. Add a small drop of 45% acetic acid and smear the material with the help of a needle. Remove debris.

3. Place a cove rs lip on the smear and warm the slide gently over the flame of a spirit lamp. Place the slide in a fold of blotting paper and apply gentle pressure.

Observe the slide under the microscope.

Following this technique the chromosome configurations were well spread and a large number of metaphase-I cells could be analysed without difficulty.

This technique, however, was not satisfactory for staining tetrads, because with the addition of acetic acid (step 2 above), the microspores of the tetrad tend to separate when the coverslip is placed on the smear.

Meiotic Characters: Metaphase-I configurations were analysed from 20 pollen mother cells (PMCs) of a plant and the frequencies of various multivalents, bivalents and univalents, chiasma frequency and disjunction index were determined. Chromosome counts were made from clear anaphase-I cells prior to metaphase-I analysis. The frequencies of "regular" metaphase-I cells (i.e. PMCs without univalents) and of "regular" tetrads (i.e. tetrads without micronuclei, bridges, polyads) were scored from each of the three anthers of a floret.

About 200 M-I cells and more than 200 tetrads were scored from each anther. For the analyses of regular tetrads, two florets from different regions of the spike were used. Thus six anthers and more than 12,00 tetrads were scored from each plant whereas only 600 M-I cells were scored per plant. Later the percentage figures were calculated for "regular" metaphase-I cells and "regular" tetrads, the percentage figures subsequently being transformed into angular values for statistical analyses (see Mather, 1951).

Vegetative Characters and Seed-set

After harvest the following observations were recorded from each plant:

1. Plant height to the base of the spike (cms)
2. Number of tillers per plant
3. Number of spikelets per spike
4. Spike length (cms)
5. Seed-set (%).

Plant height, number of spikelets, spike length and seed-set were determined from the tallest tiller of the plant. The percentage figures for seed-set were eventually transformed to angular values.

RESULTS

I. Meiotic Chromosome Associations in the Unselected Population of Tetraploid Rye

A. Chiasma Frequency

Table I.1 presents the analysis of variance for Chiasma frequency per pollen mother cells in euploid plants (genotypes) of the population.

Table I.1. Analysis of Variance for Chiasma Frequency per PMC in Plants of the Unselected Population

ITEMS	D.F.	S.S.	M.S.	F	P
Between Plants	39	491.4987	12.60253	5.344	<0.001
Error	760	1792.4503	2.35849		
Total	799	2283.9490			

The variance ratio suggests that there are significant differences between plants with regard to chiasma frequency ($P < 0.001$). In other words, a high degree of variability is present in the population. That the variation in chiasma frequency in an unselected population may largely reflect genotypic differences between plants can be concluded from several works on rye (Rees, 1955; Rees & Thompson, 1956 & 1958;

Rees, 1961; Roseweir & Rees, 1962; Jones & Rees, 1964; Hazarika & Rees, 1967). Such a feature of the population would provide scope for selection with regard to chiasma frequency. This will be examined later (Section Two).

B. Metaphase I Configurations

At metaphase-I of meiosis in an autotetraploid, the four homologues of each chromosome may be associated in a number of ways; that is as a quadrivalent (IV), as a trivalent plus a univalent (III+I), as two bivalents (2II), as one bivalent plus two univalents (II+2I) or remain unpaired as four univalents (4I). The relative frequencies of these configurations depend on the number and distribution of chiasmata within each set of homologues. It has been shown above that within the population the number of chiasmata varies significantly between plants (genotypes). When chiasma frequency is the main factor influencing the frequencies of different metaphase-I configurations, as observed by Hazarika & Rees (1967) in inbred lines, we would expect differences between plants for IV, III, II and I frequencies to correspond with the differences in chiasma frequency. These differences would indicate the existence of correlations between chiasma frequency and pairing configurations. In table I.2 the analyses of variance for different configurations are given.

Table I.2. Analyses of Variance for Different Metaphase-I Configurations in Euploid Plants of the Unselected Population

CONFIGURATIONS	ITEMS	D.F.	M.S.	F	P
IVs	Between Plants	39	1.27744	0.707	n.s.
	Error	760	1.80645		
IIIs	Between Plants	39	0.28513	1.316	n.s.
	Error	760	0.21671		
IIs	Between Plants	39	6.23641	1.027	n.s.
	Error	760	6.07237		
Is	Between Plants	39	1.20288	2.602	<0.001
	Error	760	0.46206		

n.s. = not significant

The analyses reveal that in the population none of the configurations except univalent frequency is significantly different between plants. The differences in univalent frequency explain the significant changes observed in chiasma frequency (Table I.1).

It is interesting to note that despite the significant differences between plants with regard to chiasma frequency, there are no statistically measurable changes in quadrivalent, trivalent, or bivalent frequencies. This is contrary to the findings of Roseweir & Rees (1962) and Hazarika & Rees (1967) who, in particular, demonstrated that chiasma frequency is positively correlated with quadrivalent frequency. This relationship was further examined for the material by a

regression analysis shown in table I.3.

Table I.3. Analysis of Regression of Quadrivalent Frequency on Chiasma Frequency

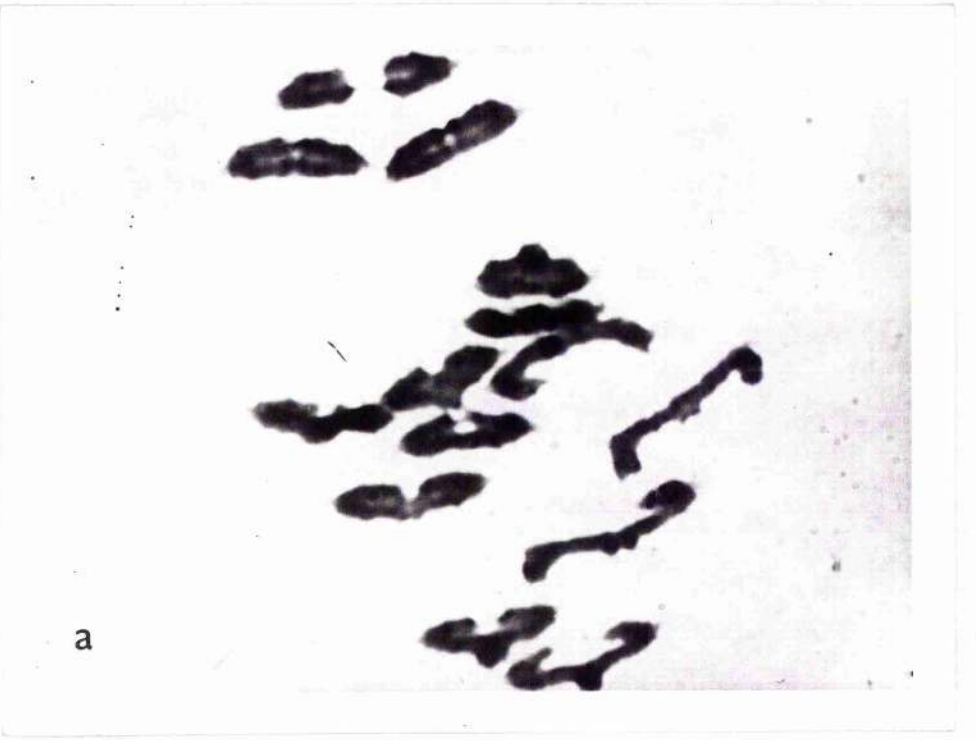
ITEM	D.F.	S.S.	M.S.	F	P
Regression	1	0.02038	0.02038	0.032	n.s.
Error	38	24.55459	0.64617		
Total	39	24.57497			

n.s. = not significant

The regression is insignificant which confirms the lack of relationship between quadrivalent frequency and chiasma frequency. This is also illustrated in Plate I.1. where it will be noticed that the progression in quadrivalent frequency is not necessarily related with chiasma frequency.

C. Disjunction Index

Hazarika & Rees (1967) suggested an index by which the proportion of PMCs giving equal chromosome disjunction at anaphase-I can be estimated. They gave the name "Disjunction Index" to the proportion of PMCs without trivalents and univalents, the configurations normally responsible for irregular chromosome separation. The disjunction index (D.I.), along with other meiotic properties, of the individual plants have been shown in the appendix (Appendix Table 10). Hazarika & Rees (1967) showed that D.I. has a strong positive



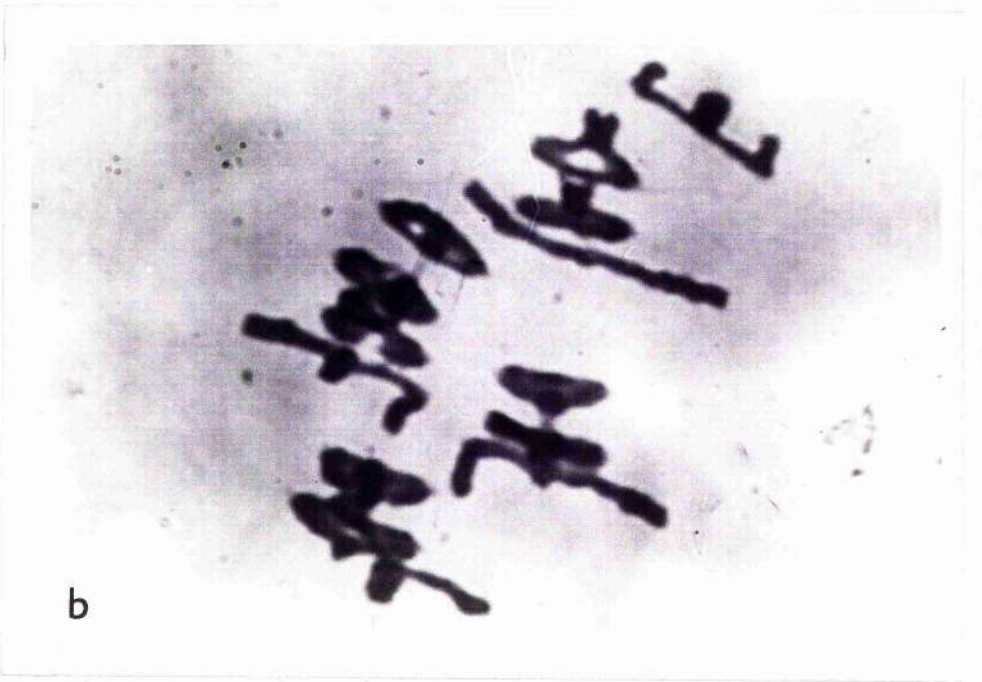
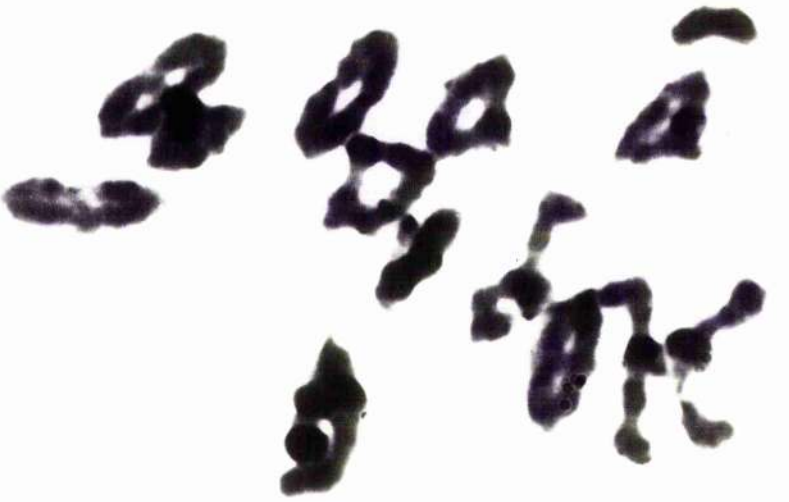


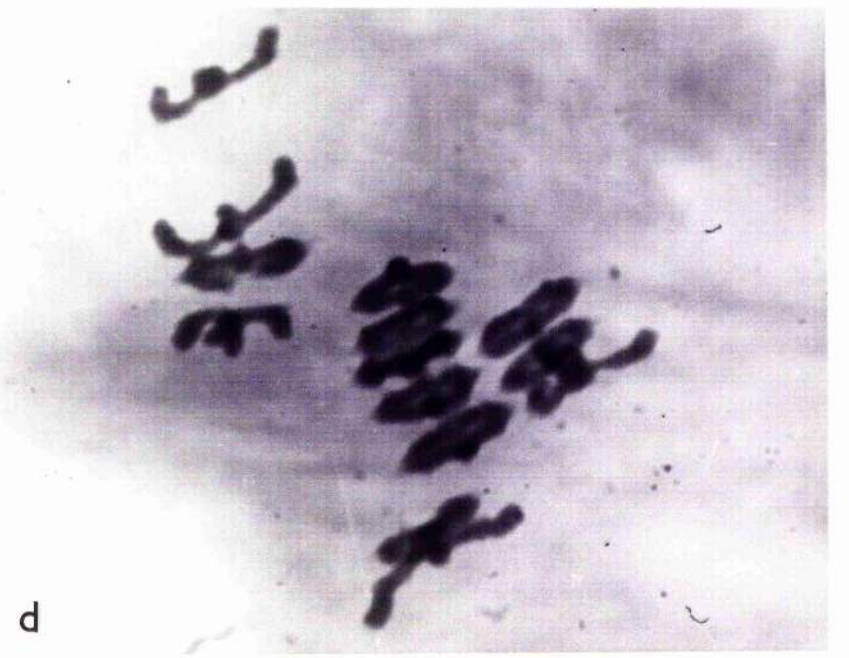
PLATE I.1. M-I CONFIGURATIONS

a - 14^{II}, Xta.≙25; b - 14^{II}, Xta.≙23



c

10 µm

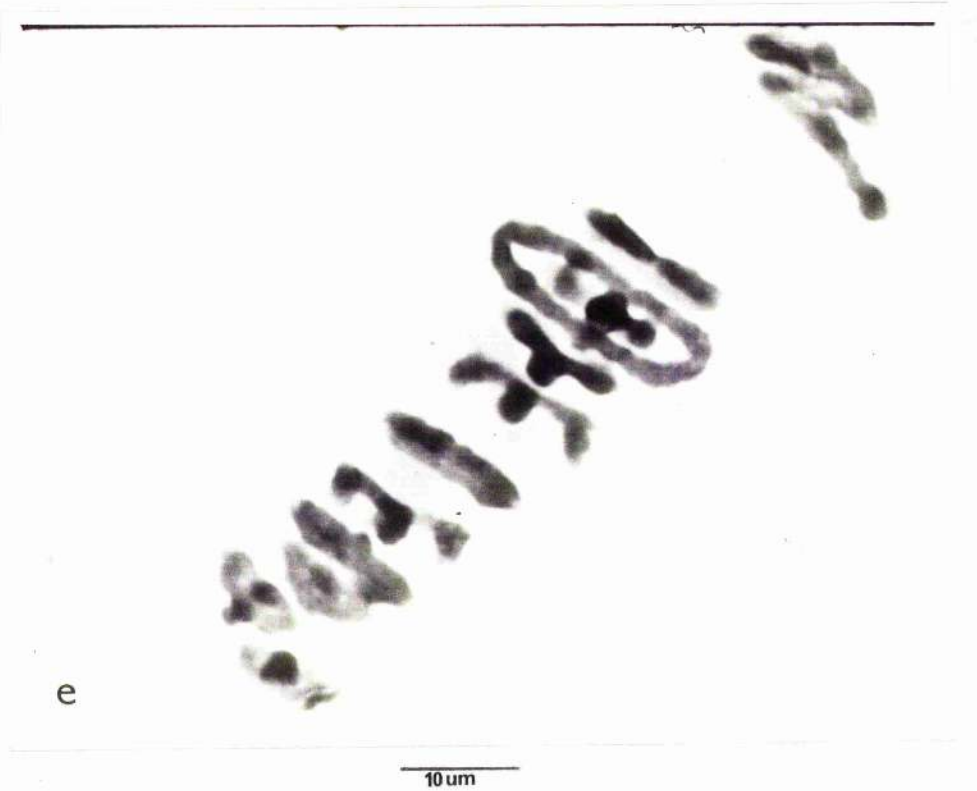


d

10 µm

PLATE I.1. M-I CONFIGURATIONS (Contd.)

c - 1^{III} 12^{II} 1^{I}, Xta.≏23; d - 1^{IV} 12^{II}, Xta.≏23



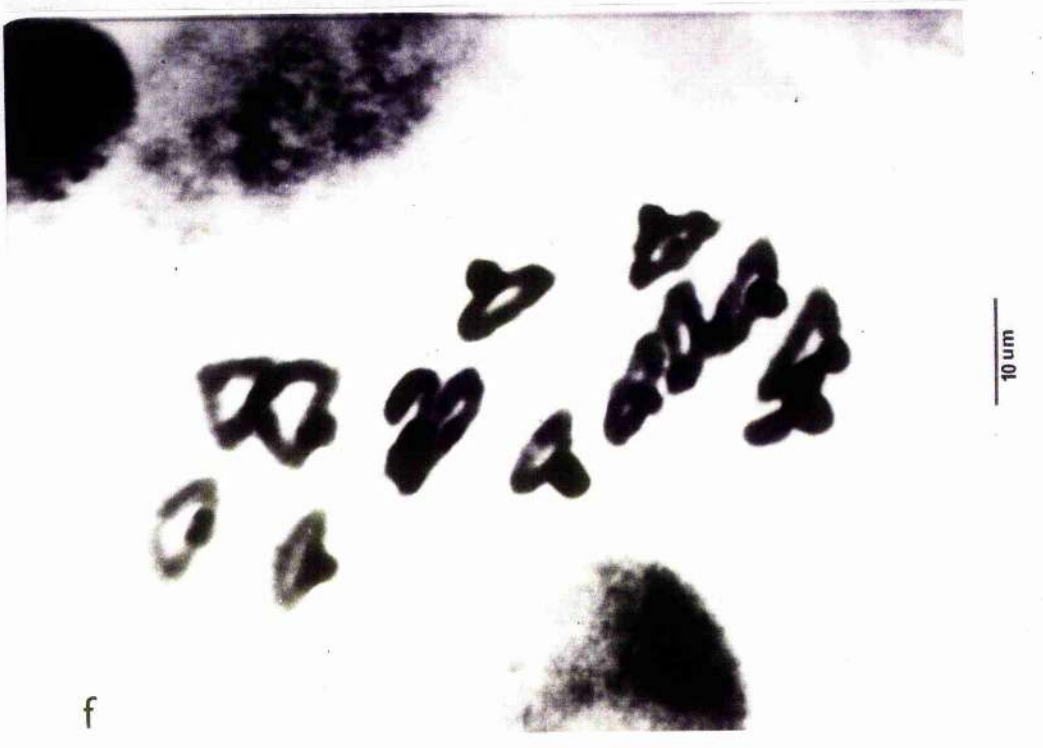
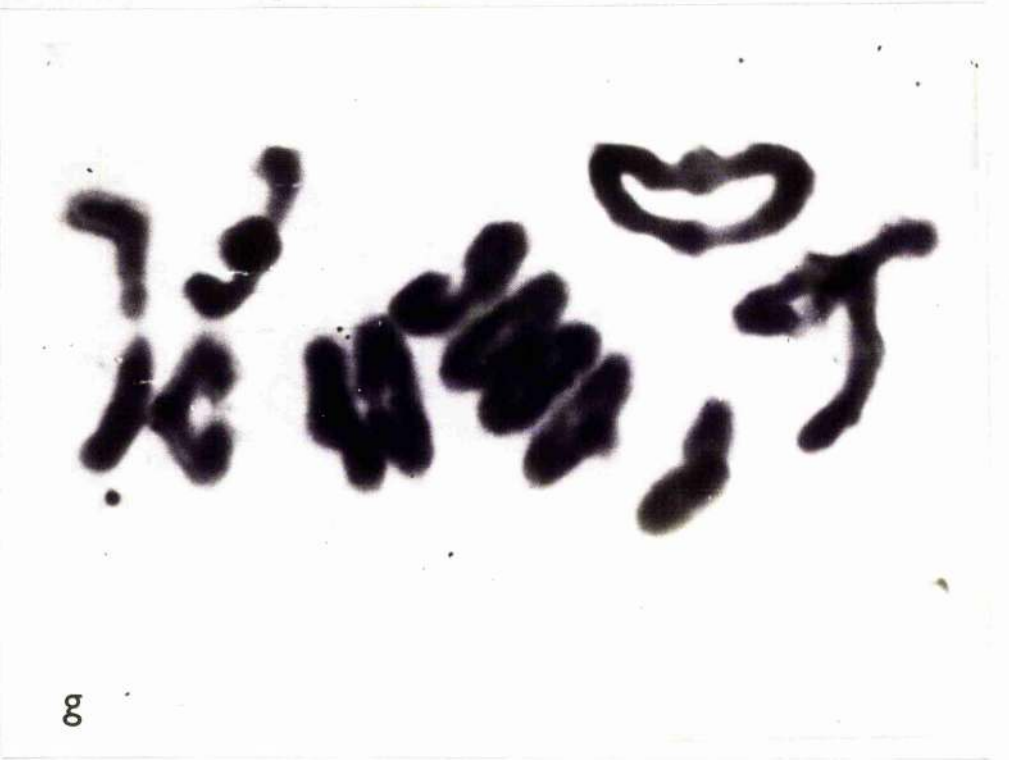


PLATE I.1. M-I CONFIGURATIONS (Contd.)

e - 2^{IV} 10^{II}, Xta. ~24; f - 2^{IV} 10^{II}, Xta. ~27



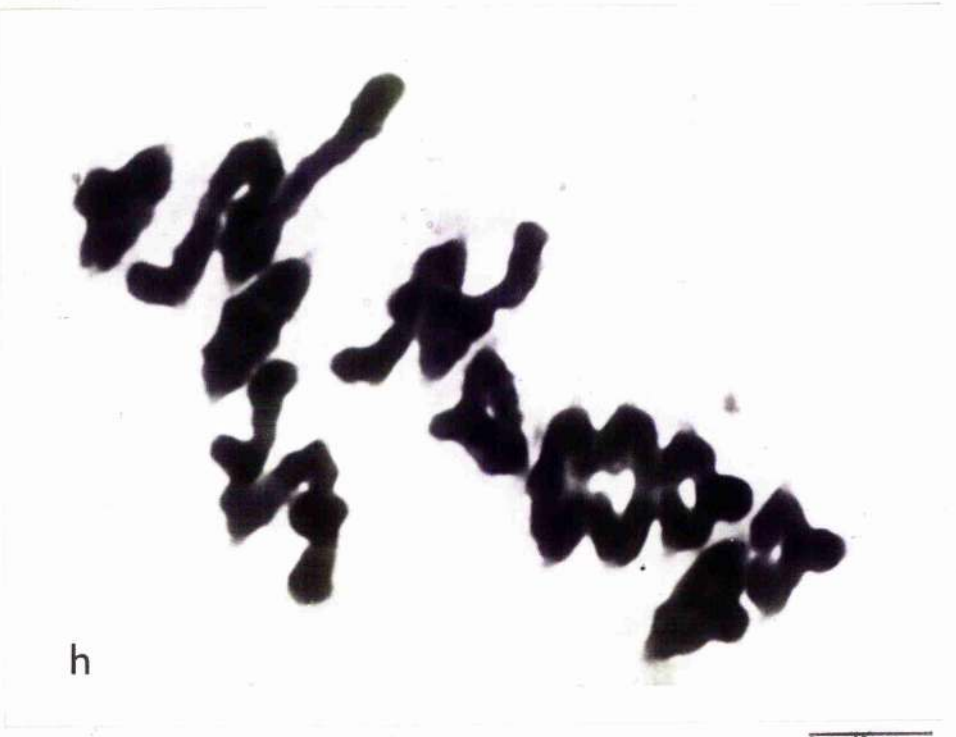


PLATE I.1. M-I CONFIGURATIONS (Contd.)

g - 3^{IV} s^{II}, Xta.≏25; h - 3^{IV} s^{II}, Xta.≏25

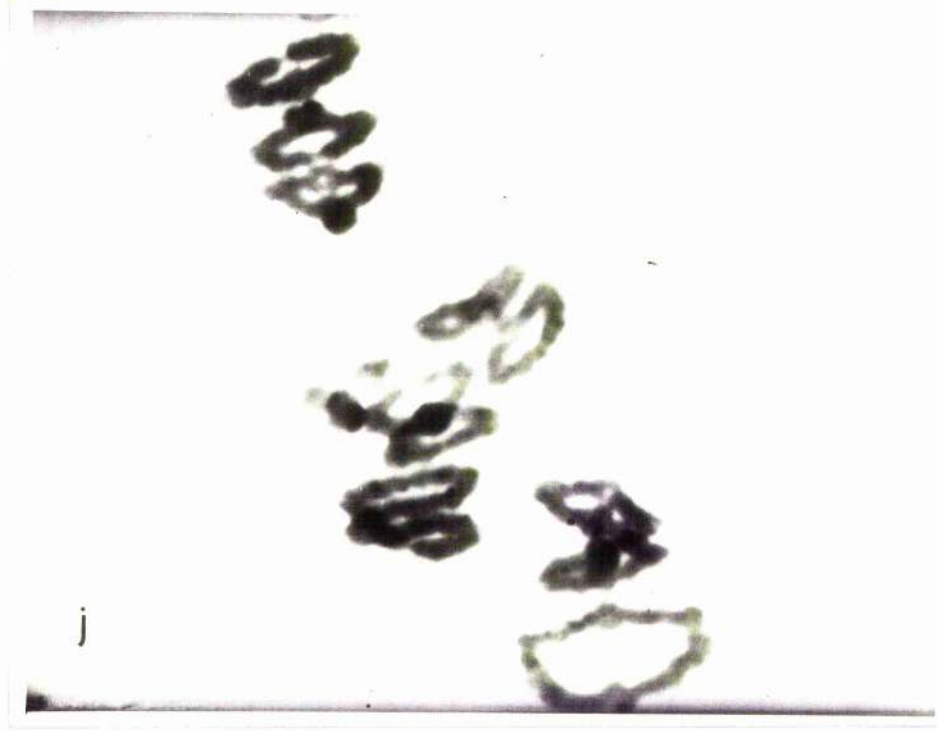
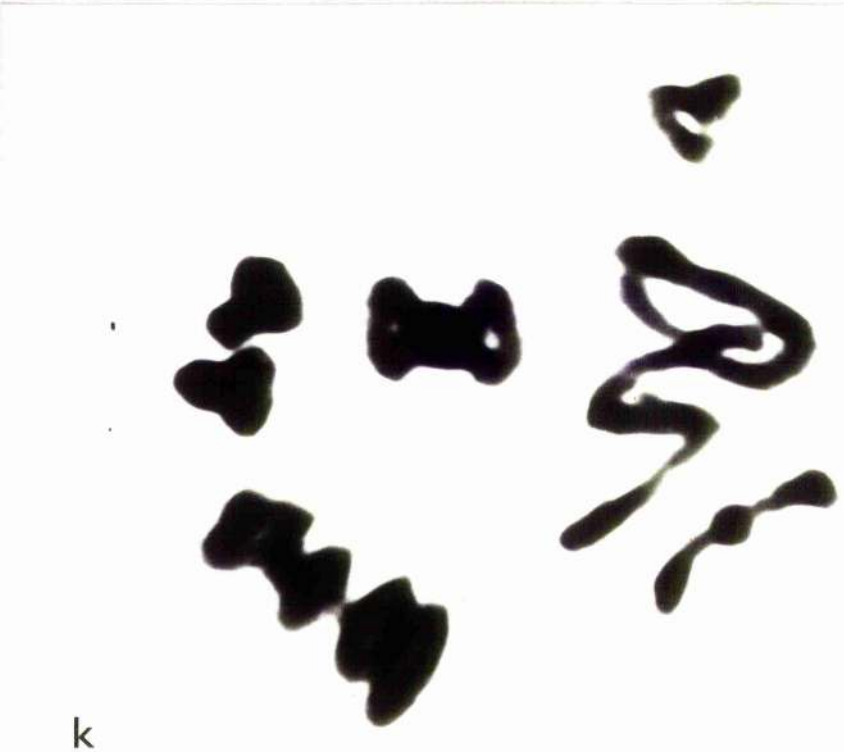


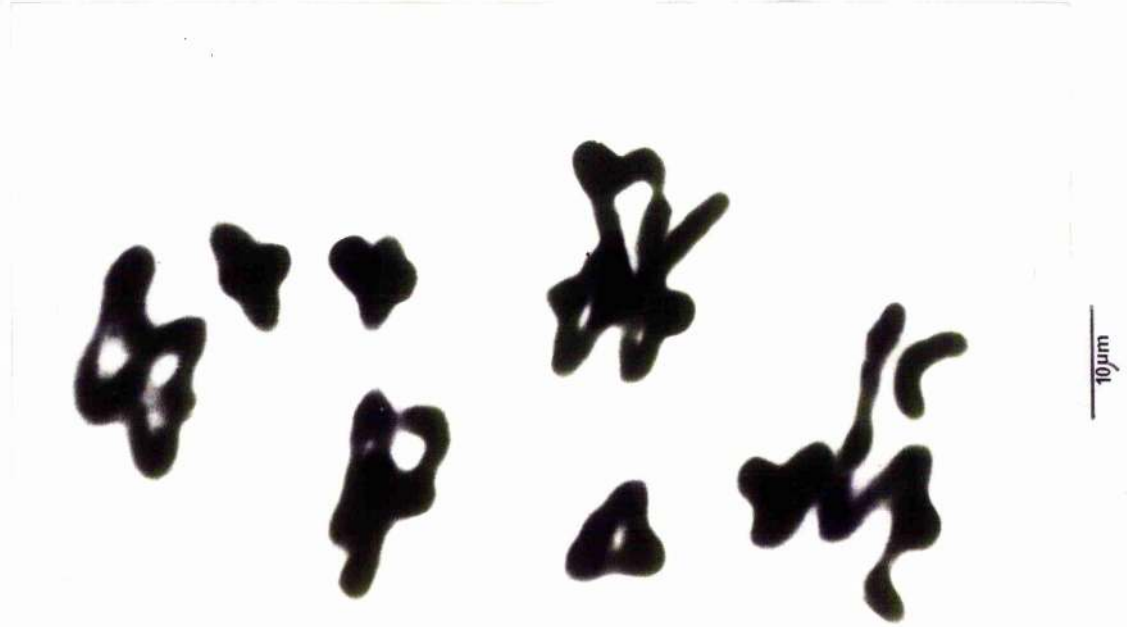
PLATE I.1. M-I CONFIGURATIONS (Contd.)

i - 3^{IV} 8^{II}, Xta.≏26; j - 3^{IV} 8^{II}, Xta.≏28



k

10 µm



l

10µm

PLATE I.l. M-I CONFIGURATIONS (Contd.)

k - 4^{IV} 6^{II}, Xta.≏26; l - 4^{IV} 1^{III} 4^{II} 1^{I}, Xta.≏25

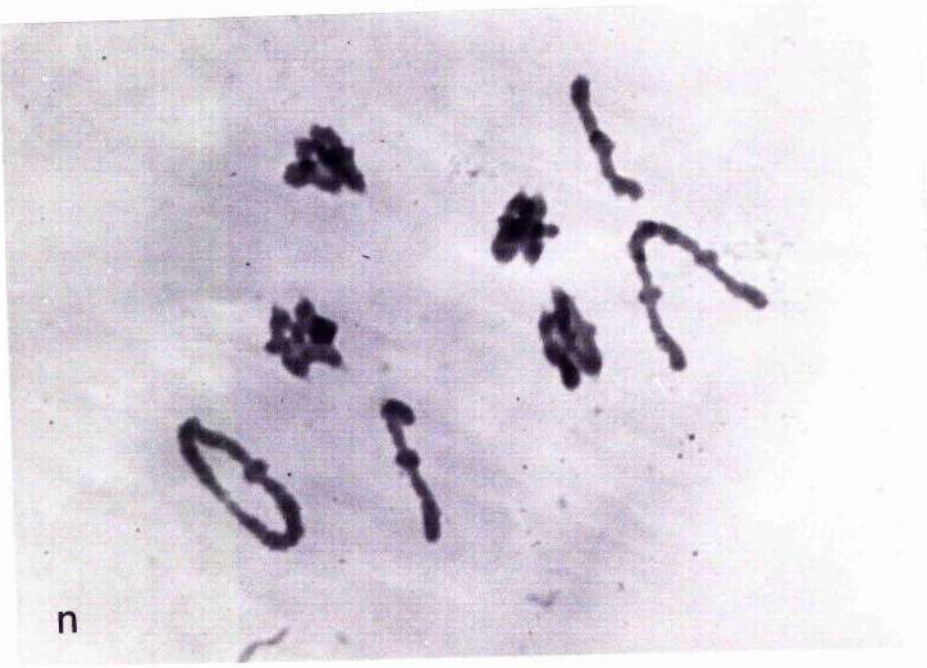
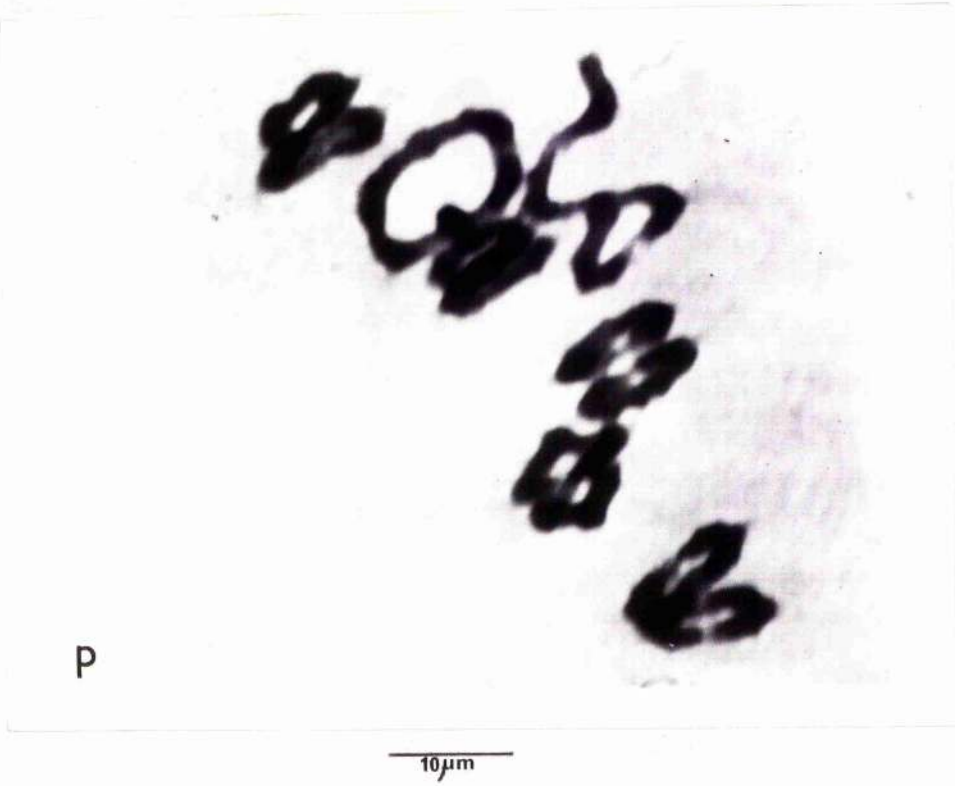


PLATE I.1. M-I CONFIGURATIONS (Contd.)

m - 5^{IV} 4^{II}, Xta.≏28; n - 6^{IV} 2^{II}, Xta.≏25



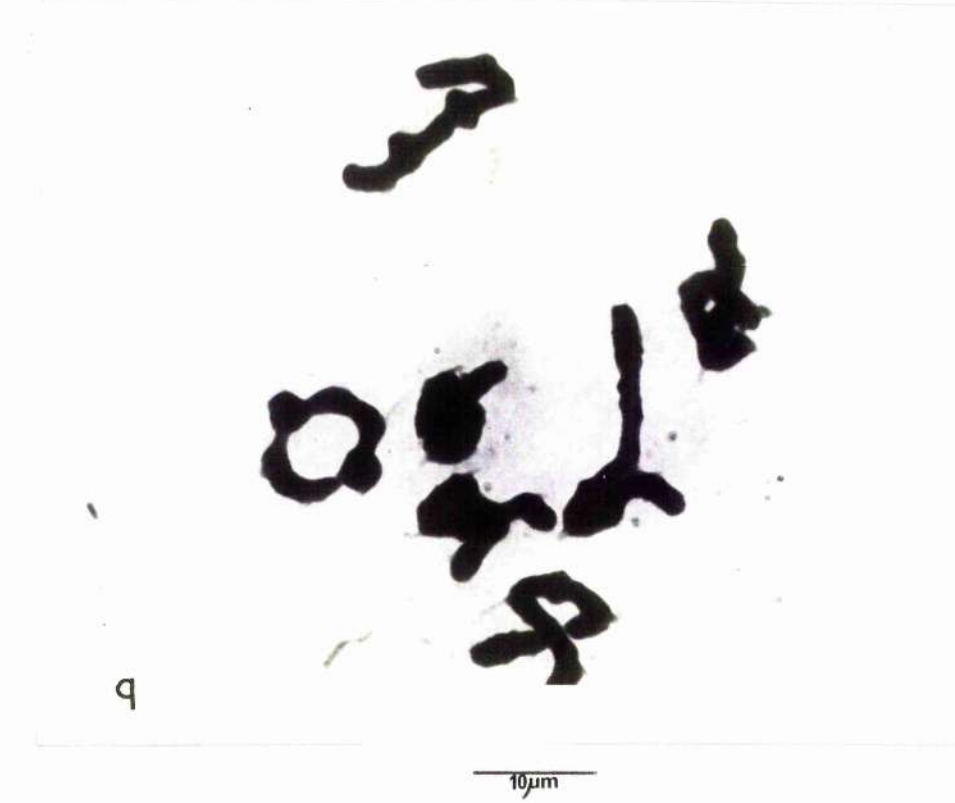


PLATE I.1. M-I CONFIGURATIONS (Contd.)

p - 6^{IV} 2^{II}, Xta.≙23; q - 7^{IV} , Xta.≙22

correlation with quadrivalent frequency as well as fertility and emphasised the significance of the index in breeding autotetraploids, rye in particular. This will be examined for the unselected population in the next section.

D. Regular Metaphase-I Cells

Disjunction Index is a tedious method of estimating meiotic regularity involving detailed metaphase-I analysis. A relatively simple method of estimating regularity would be by screening PMCs (using a magnification $\times$ 400) for the presence of univalents at metaphase-I. By this method several hundreds of PMCs can be scored quickly for a number of anthers, each acting as a replicate. These replicated observations provide data suitable for statistical analysis as shown in table I.4. Despite the possibility of missing some univalents, the increase in sample size improves the accuracy of the estimate of meiosis regularity for a plant.

Table I.4. Analysis of Variance for Regular Metaphase-I Cells (Angular Values) in Euploid Plants of the Unselected Population

ITEMS	D.F.	S.S.	M.S.	F	P
Plants	39	3890.1450	99.7473	15.566	<0.001
Error	80	512.6537	6.40817		
Total	119	4402.7987			

The table shows highly significant differences between plants for the proportion of regular M-I cells ($P<0.001$). This was expected in view of the differences observed earlier for chiasma frequency and univalent frequency. Since the differences observed for chiasma frequency were considered to be genetical in origin, the differences between plants for regular metaphase-I cells must also be of genetic origin, because, as will be shown later, chiasma frequency is strongly correlated with the proportion of regular metaphase-I cells.

E. Regular Tetrads

Another simple but quick method of estimating the degree of meiotic regularity is by scoring regular tetrads as opposed to tetrads with micronuclei. The micronuclei in tetrads are in fact derived from the univalents at metaphase I. This is illustrated in plate I.2. Regular tetrad, therefore, serves as an alternative to regular metaphase-I cell for determining meiosis regularity and for that matter, it is also an alternative to disjunction index. The duration of the tetrad stage is one of the longest among the several meiotic stages (Bennet et al., 1971) and a number of florets from different regions of the spike can be obtained at this stage at the time of fixation. Plant means obtained from a number of samples taken from different regions of the spike can take into account the variations, developmental or environmental (Walther, 1959; Rees & Naylor, 1960) that may exist within the spike.

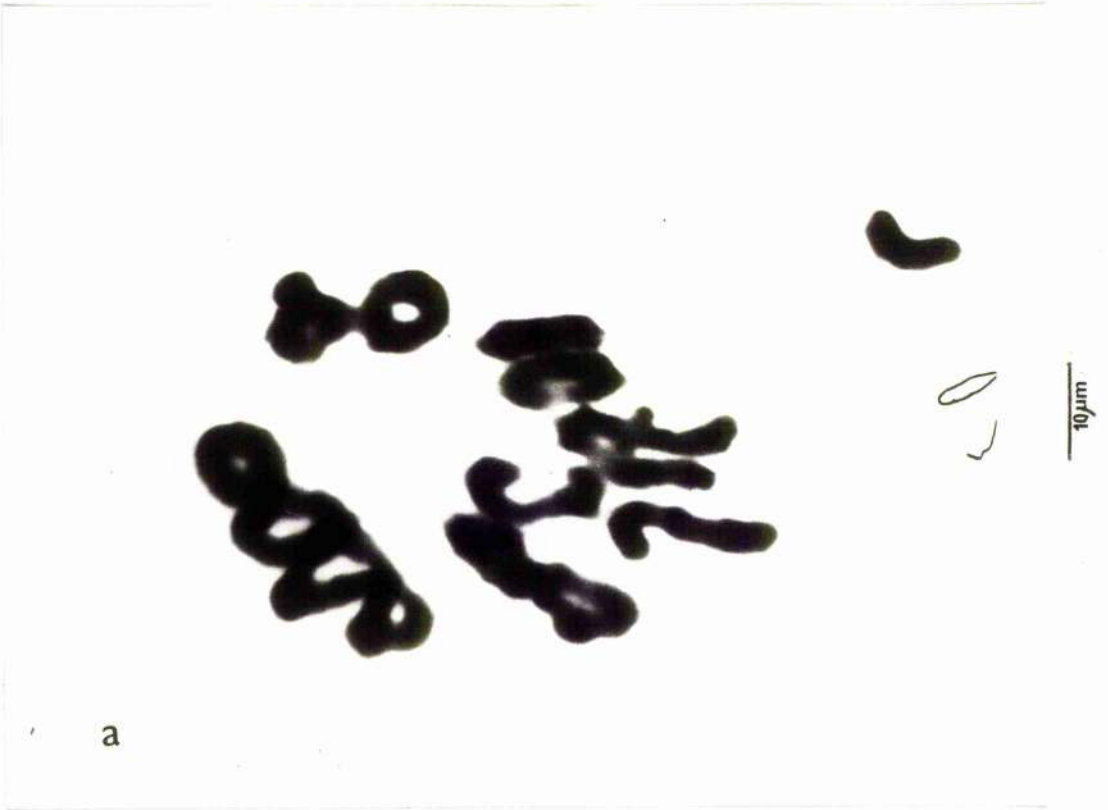


PLATE I.2. UNIVALENTS AT MEIOSIS

a - M-I showing one univalent; b - M-I showing two univalents.

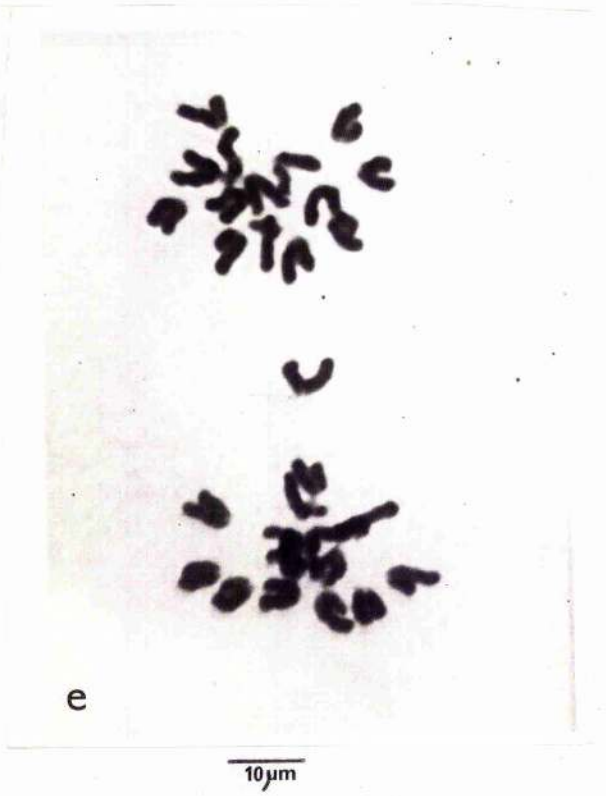
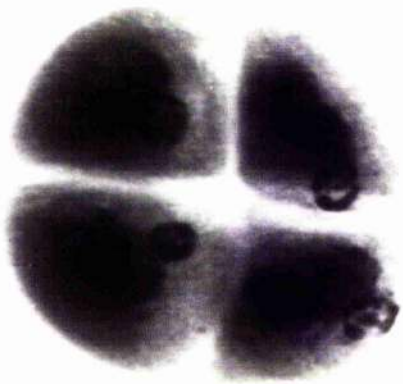


PLATE I.2. UNIVALENTS AT MEIOSIS (Contd.)

c - A-I showing two univalents separating. Note delayed separation of univalents; d - M-II showing two chromatids as laggards; e & f - A-II showing laggards.

g



h

PLATE I.2. UNIVALENTS AT MEIOSIS (Contd.)

g - T-II showing four lagging chromatids; h - Tetrad showing four micronuclei derived from four laggards at T-II.

The existence of such variations within the spike was detected in the present material. For this 5 florets each with replicated observations from the three anthers were analysed. Five plants were used for the analysis. The results are shown in table I.5.

Table I.5. Analysis of Variance for the Proportion of Regular Tetrads Using Five Florets per Plant and Three Anthers per Floret

ITEMS	D.F.	S.S.	M.S.	F	P
Between Plants	4	408.82	102.21	30.60	<0.001
Between Florets	4	179.77	44.94	10.13	<0.001
Interaction: (Plant X Floret)	16	88.59	5.54	1.66	n.s.
Between Anthers (Error)	50	167.29	3.34		

n.s. = not significant

The table shows highly significant differences between plants ($P<0.001$) as well as between florets within the plant ($P<0.001$). While the differences between plants were expected, between floret differences demonstrate the variations which are developmental or environmental in origin. The latter makes it necessary to include a sufficient number of observations from a plant to get a reliable estimate of the plant mean. This was computed from the same original data used in the analysis in table I.5 as follows (Sokal & Rohlf, 1969).

Table I.6. Analysis of Variance

ITEMS	D.F.	S.S.	M.S.	Parameters
Between Plants	4	408.82	102.19	$\sigma^2_e+3\sigma^2_{fl}+15\sigma^2_{pl}$
Between Florets within Plants	20	268.36	13.41	$\sigma^2_e+3\sigma^2_{fl}$
Between Anthers within Florets within Plants	50	167.27	3.34	σ^2_e

From the table, the variances due to different items are:

(i) Error Variance (MS_e) = 3.34

(ii) Variance of Floret Mean = $\frac{MS_{fl} - MS_e}{\text{No. of anthers in each floret}}$

$= \frac{13.41 - 3.34}{3} = 3.36$

(iii) Variance of Plant Mean = $\frac{MS_{pl} - (MS_e+3MS_{fl})}{\text{No. of anthers in each plant}}$

$= \frac{102.19 - (3.34 + 3 \times 3.36)}{15}$

$= 5.91$

Now,

(a) With 5 florets/plant and 3 anthers/floret, the Expected Variance of Plant Mean is given by

$$S^2_{\bar{y}(a)} = \frac{S^2_e}{n.b} + \frac{S^2_{fl}}{b} \quad \text{where} \quad n = \text{no. of plants}, \; b = \text{no. of florets}$$

$$\therefore \; S^2_{\bar{y}(a)} = \frac{3.34}{3 \times 5} + \frac{3.36}{5} = 0.89$$

(b) With a reduction in the number of florets from 5 to 2, the Expected Variance of Plant Mean

$$S^2_{\bar{y}(b)} = \frac{3.34}{3 \times 2} + \frac{3.36}{2} = 1.90$$

The relative efficiency of design (a) with respect to design (b) is given by

$$\text{R.E.} = \frac{S^2_{\bar{y}(b)}}{S^2_{\bar{y}(a)}} = \frac{1.90}{0.89} = 2.135$$

If the no. of florets in the model design (i.e. a) is divided by R.E., the resulting design would be as efficient as the model design (Sokal & Rohlf, 1969).

$$\therefore \; \text{The Required No. of Florets} = \frac{5}{2.135} = 2.3 \text{ Florets.}$$

It was, therefore, decided that in order to get a reliable plant mean, at least 2 florets with 3 anthers in each should be analysed for the proportion of regular tetrads.

Based on data obtained from two different florets the analysis of variance in table I.7 shows that regular tetrads, like chiasma frequency and regular metaphase-I cells, vary significantly between plants.

Table I.7. Analysis of Variance for Regular Tetrads (Angular Values) in Euploid Plants of the Unselected Population

ITEMS	D.F.	S.S.	M.S.	F	P
Between Plants	39	5117.706	131.2232	5.208	<0.001
Error	200	5039.328	25.1966		
Total	239	1057.034			

That the variations between plants with regard to regular tetrads are also genetic in origin can be envisaged from the fact that irregular tetrads are identified by the presence of micronuclei, the latter being derived from univalents at metaphase-I which in turn depends on chiasma frequency (Roseweir & Rees, 1962; Hazarika & Rees, 1967). Suffice it to say here that the variations in regular tetrads, like chiasma frequency and disjunction index can also be used for selection purpose and this was in fact attempted in the present investigation (Section Two).

II. Inter-relationships of Meiotic Properties

The correlation coefficients of different meiotic properties are given in table I.8.

Chiasma frequency is significantly correlated with all other meiotic properties except quadrivalent frequency. The lack of relationship of chiasma frequency with quadrivalents will be undertaken later for discussion. However, when quadrivalent frequency was combined with bivalent frequency (IV+II), the total number of chromosomes was significantly correlated with chiasma frequency ($r_{(38)} = 0.670$; $P < 0.001$). This was expected because the number of chromosomes involved in IV_8+II_8 is measured against the number of chromosomes in trivalents and univalents (III_8+I_8), the latter configurations being normally associated with reduced chiasma frequency. This was confirmed by the negative correlation of chiasma frequency with trivalent as well as univalent frequency (Figures I.1 & I.2). A similar relationship can be observed when the total number of chromosomes in trivalents and univalents is correlated with chiasma frequency ($r_{(38)} = -0.670$; $P < 0.001$). Chiasma frequency is positively correlated with bivalent frequency (Figure I.3), disjunction index (Figure I.5), proportion of regular metaphase-I cells (Figure I.6) and of regular tetrads (Figure I.7).

Table I.8. Correlation Coefficients of Different Meiotic Features in the Unselected Population (Euploids only).

Characters	Cell-variance for chias-mata	IV Frequency	III Frequency	II Frequency	I Frequency	No. of chromo-somes in (IV+II)	No. of chromo-somes in (III+I)	Dis-junction Index	Regular MI Cells	Regular Tetrads
Chiasma Frequency	-0.271	0.029	-0.565***	0.313*	-0.736***	0.670***	-0.670***	0.632***	0.678***	0.662***
Cell-variance for chiasmata	-	-0.070	-0.087	-0.070	0.097	0.014	-0.014	-0.028	-0.032	-0.085
IV Frequency		-	-0.028	-0.863***	-0.200	0.123	-0.123	0.110	0.041	0.004
III Frequency			-	-0.466***	0.804***	-0.960***	0.960***	-0.896***	-0.857***	-0.544***
II Frequency				-	-0.289*	0.389**	-0.389**	0.383**	0.414**	0.296*
I Frequency					-	-0.909***	0.909***	-0.919***	-0.823***	-0.588***
No. of chromo-somes in (IV+II)						-	-1.000***	0.931***	0.881***	0.594***
No. of chromo-somes in (III+I)							-	-0.931***	-0.881***	-0.594***
Disjunction Index								-	0.815***	0.554***
Regular MI Cells									-	0.685***

n = 40

* indicates significant at 5% level
** " " " 1% "
*** " " " 0.1% "

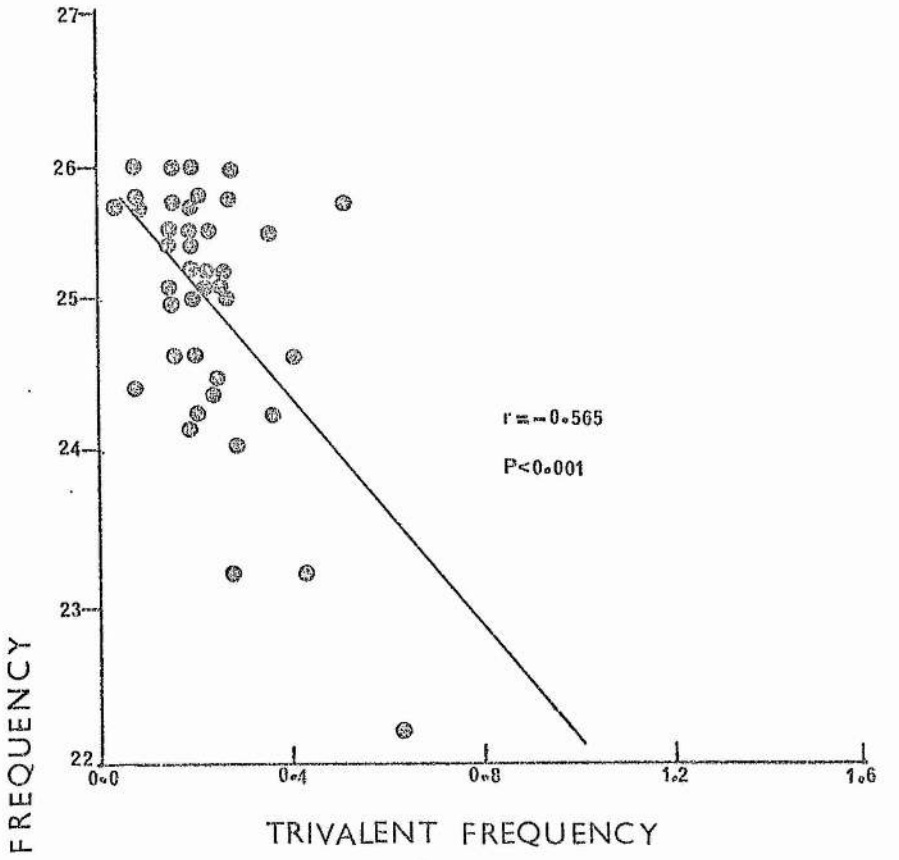


Figure 1.1

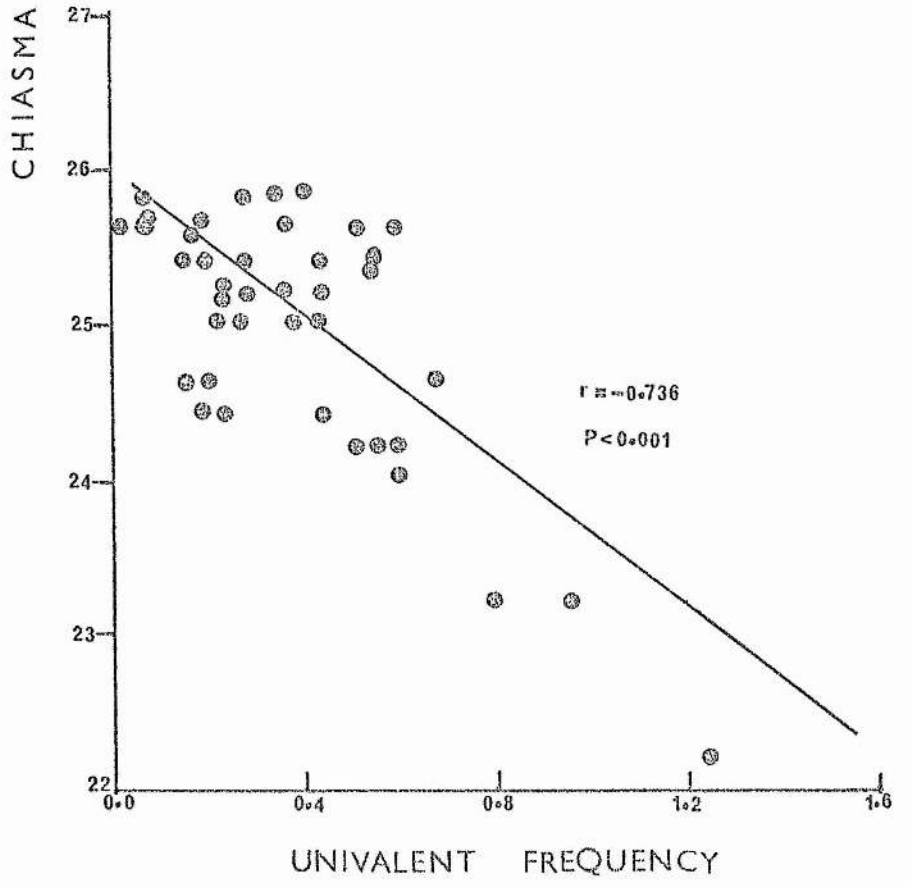


Figure 1.2

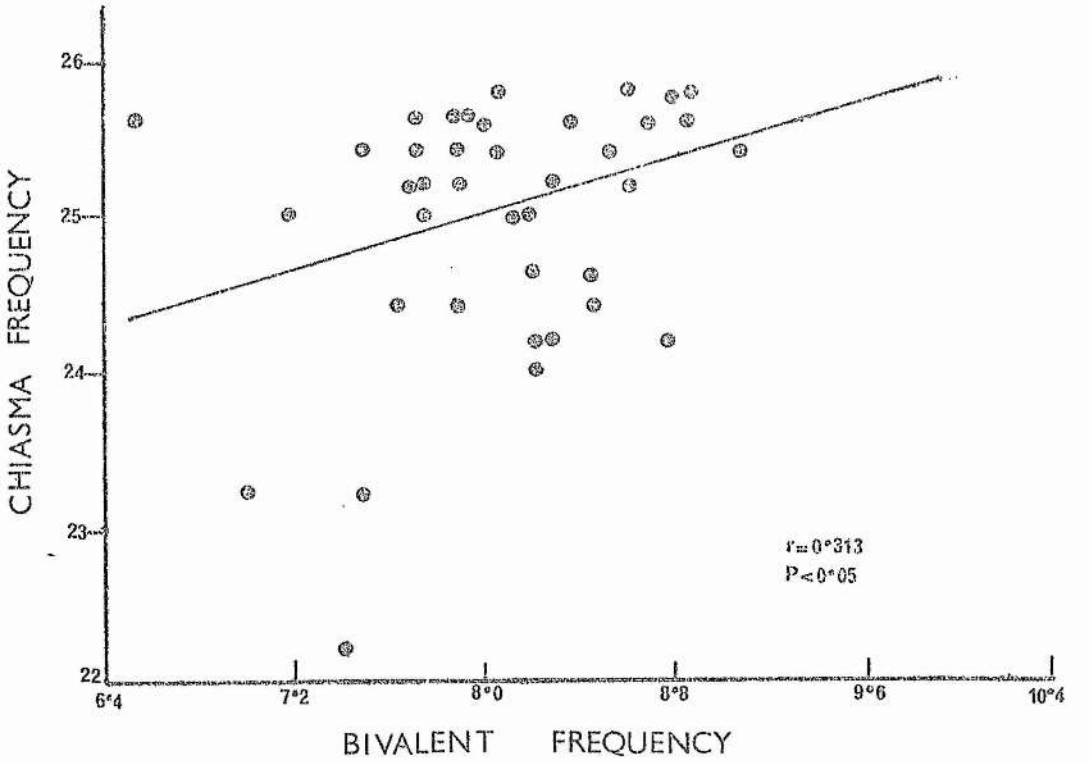

CHIASMA FREQUENCY
26
25
24
23
22
r=0·313
P<0·05
6·4
7·2
8·0
8·8
9·6
10·4
BIVALENT FREQUENCY

FIG. 1.3

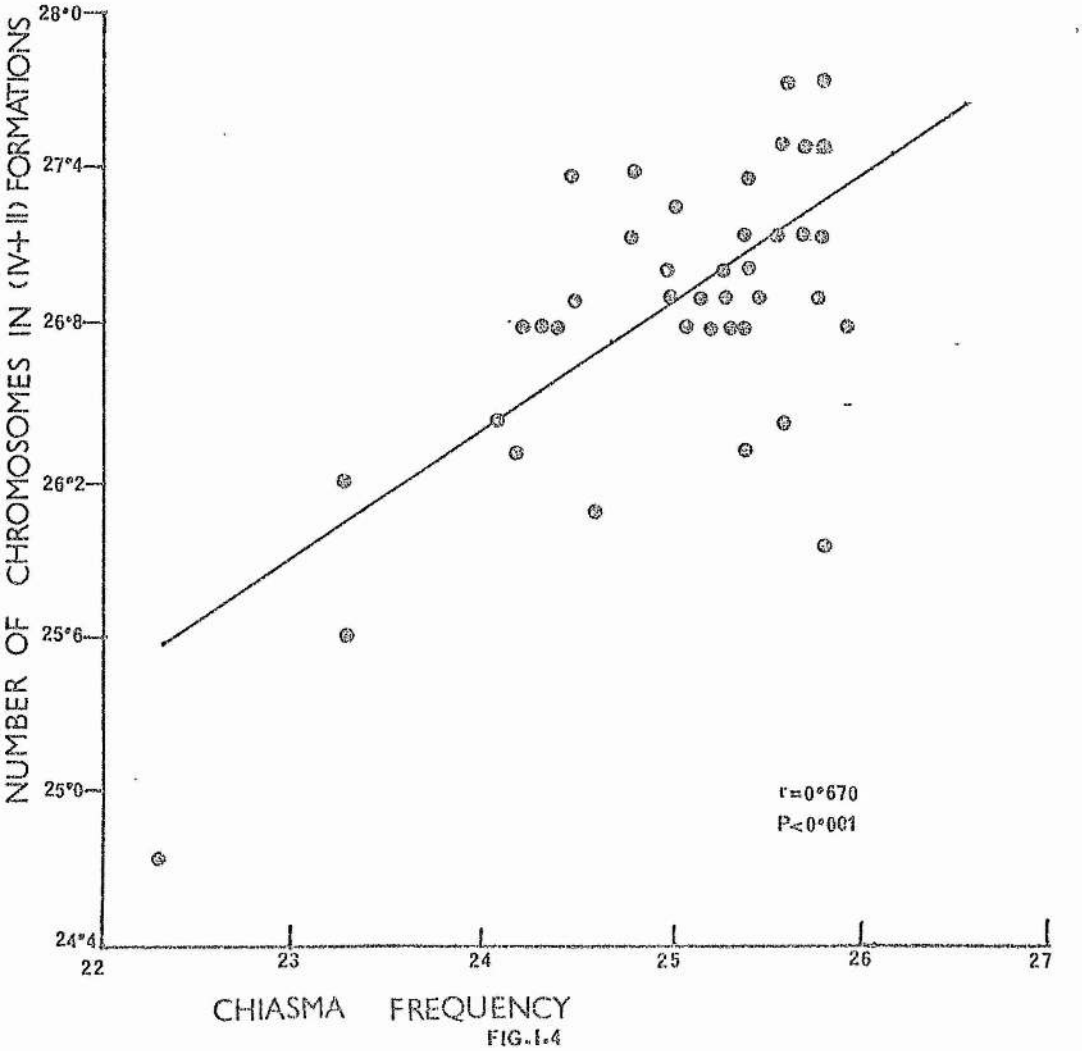

NUMBER OF CHROMOSOMES IN (IV+II) FORMATIONS
28·0
27·4
26·8
26·2
25·6
25·0
24·4
r=0·670
P<0·001
22
23
24
25
26
27
CHIASMA FREQUENCY

FIG. 1.4

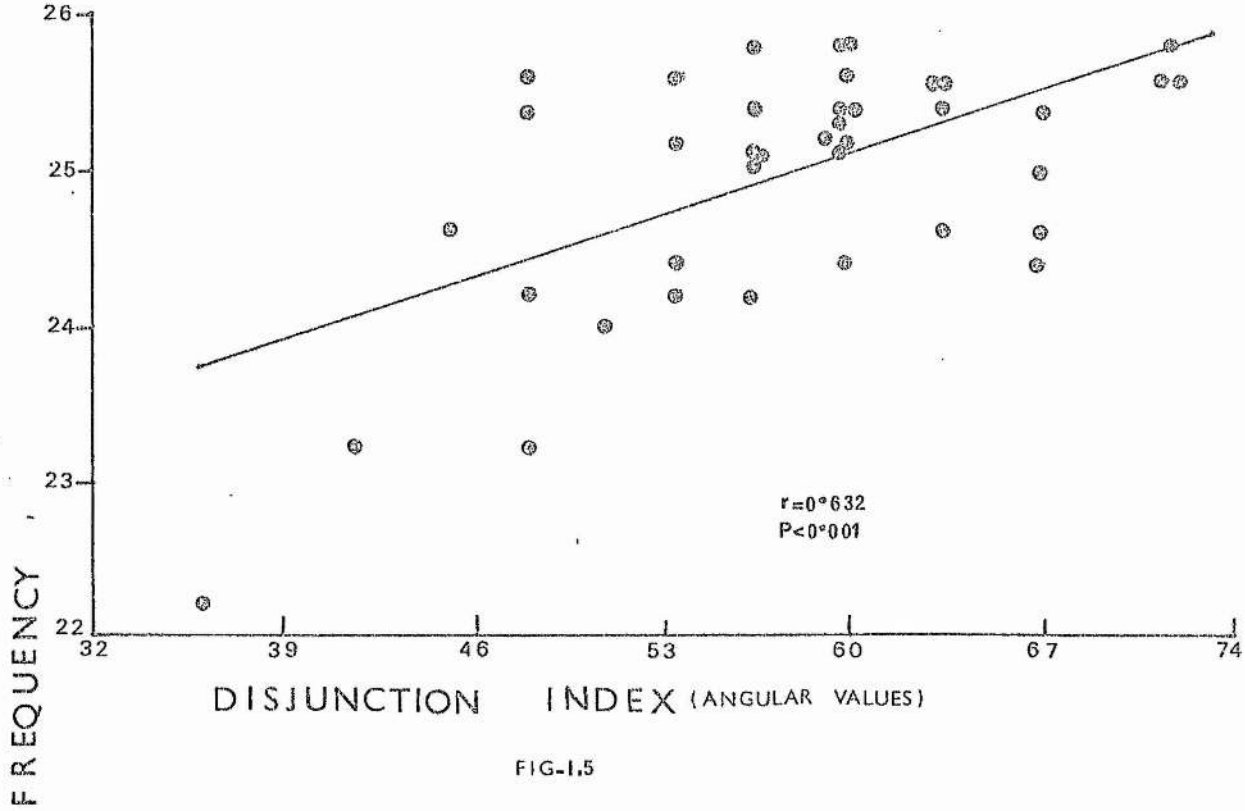

26
25
24
23
22
FREQUENCY
r=0°632
P<0°001
32
39
46
53
60
67
74
DISJUNCTION INDEX (ANGULAR VALUES)

FIG-1.5

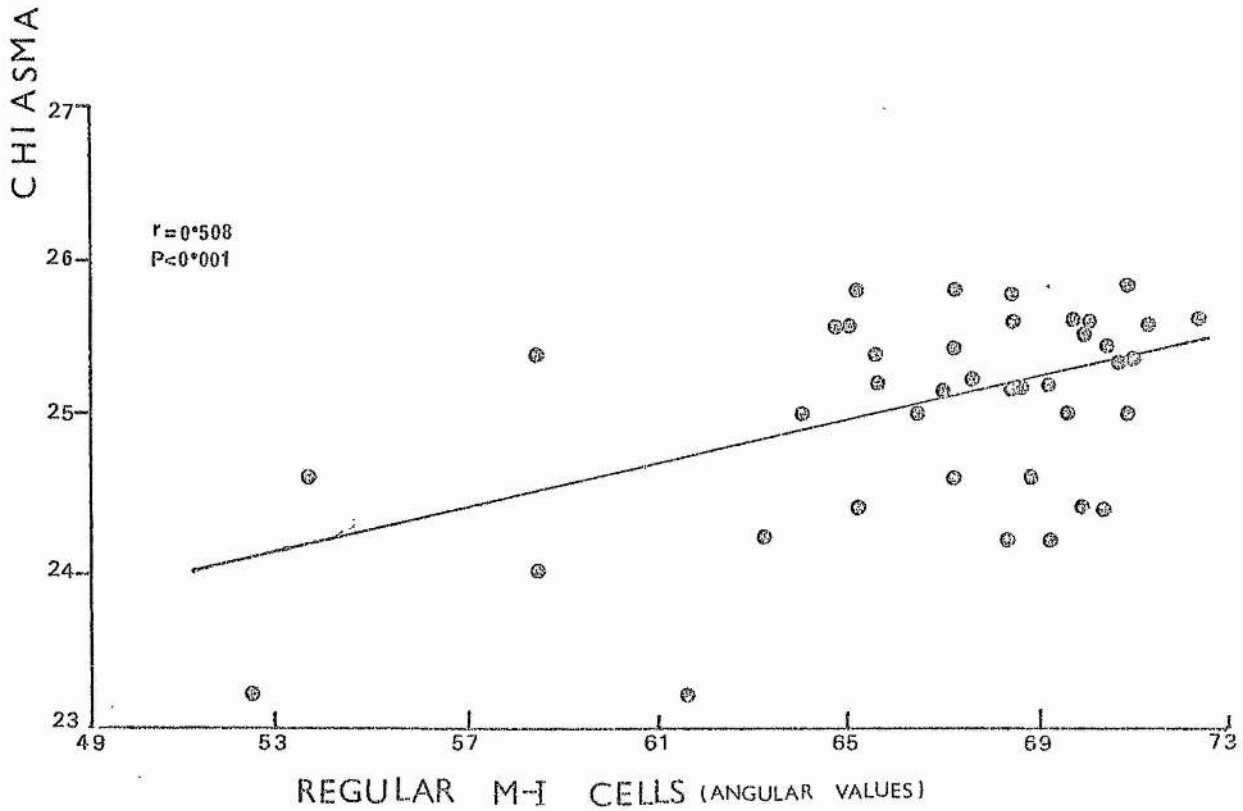

CHIASMA
27
26
25
24
23
r=0°508
P<0°001
49
53
57
61
65
69
73
REGULAR M-I CELLS (ANGULAR VALUES)

FIG-1.6

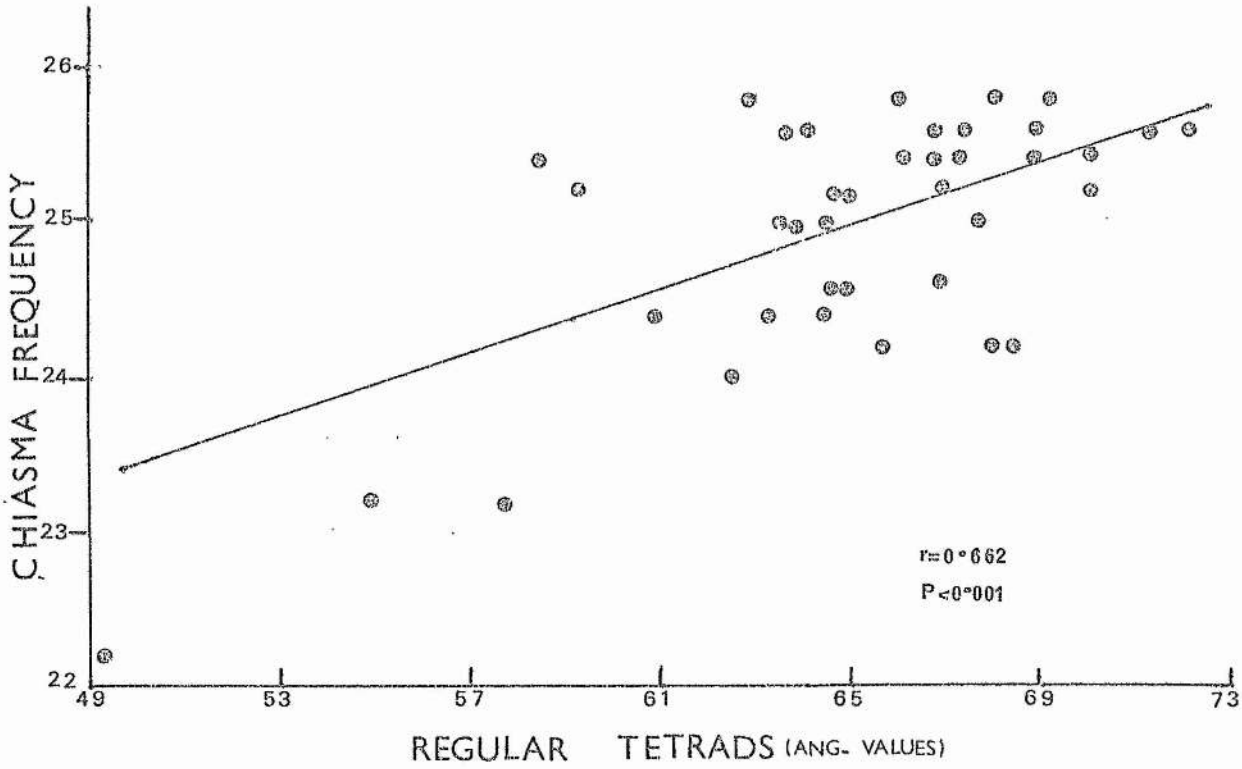

CHIASMA FREQUENCY
26
25
24
23
22
49
53
57
61
65
69
73
r=0·662
P<0·001
REGULAR TETRADS (ANG. VALUES)

FIG. 1.7

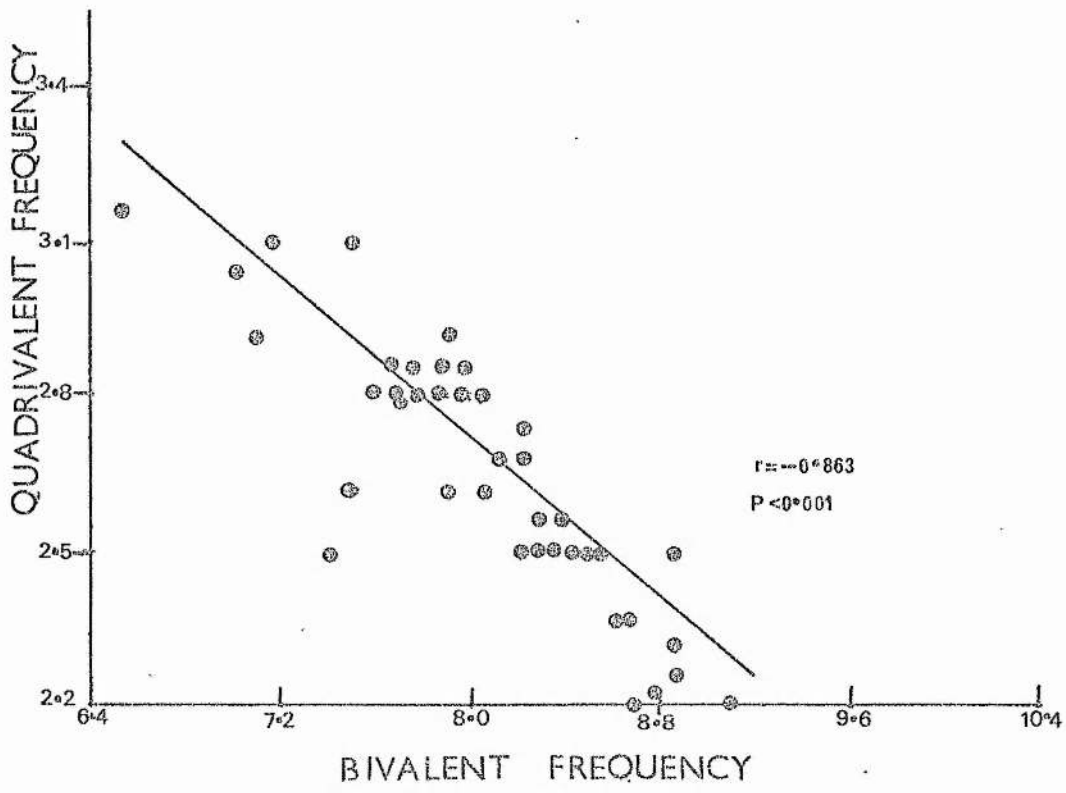

QUADRIVALENT FREQUENCY
3·4
3·1
2·8
2·5
2·2
6·4
7·2
8·0
8·8
9·6
10·4
r=-0·863
P<0·001
BIVALENT FREQUENCY

FIG. 1.8

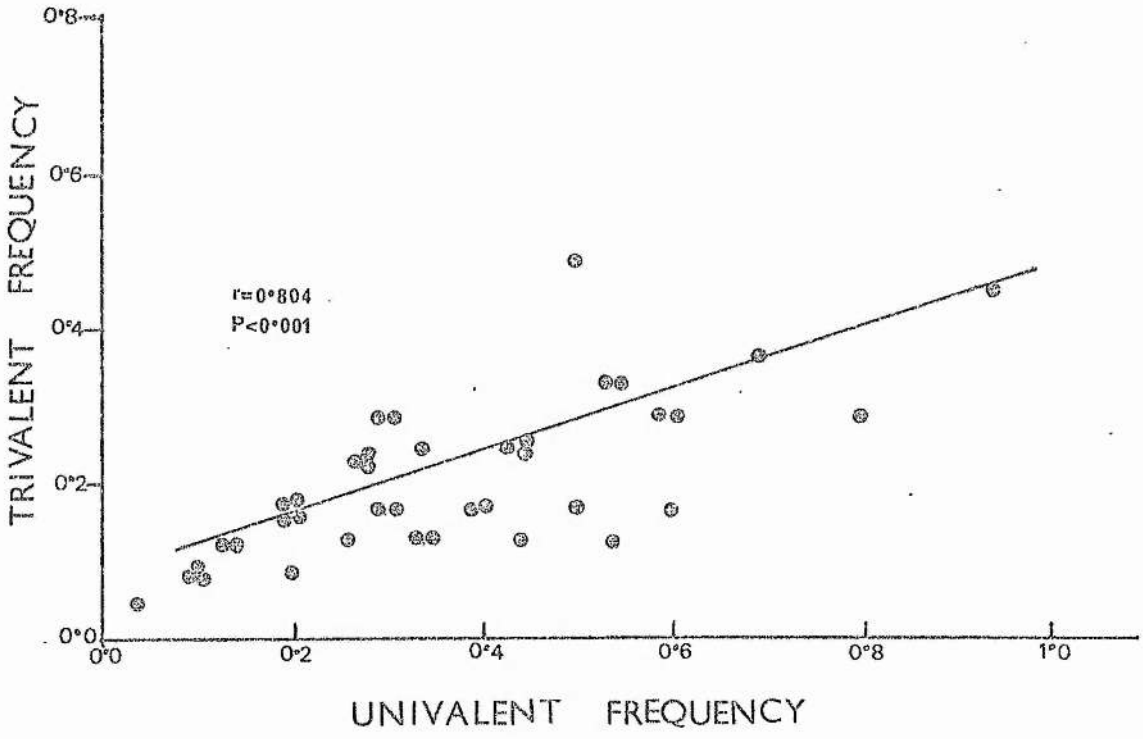


FIG.I.9

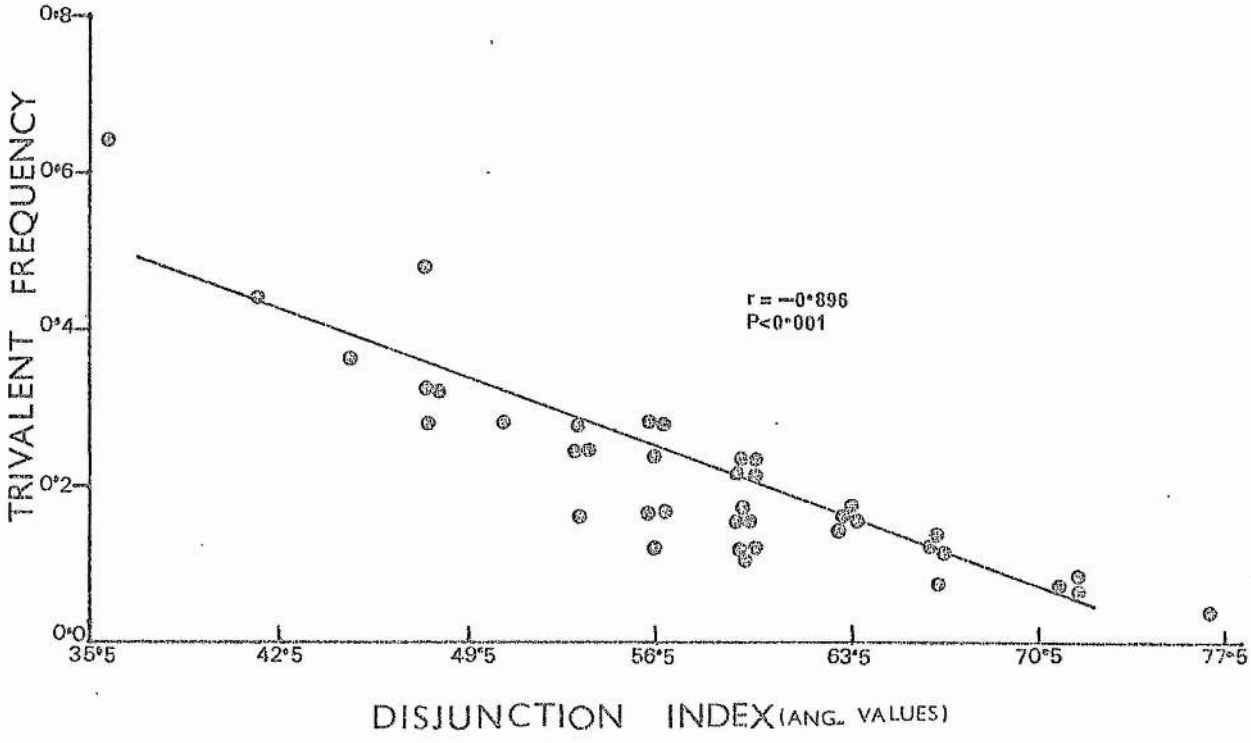


FIG.I.10

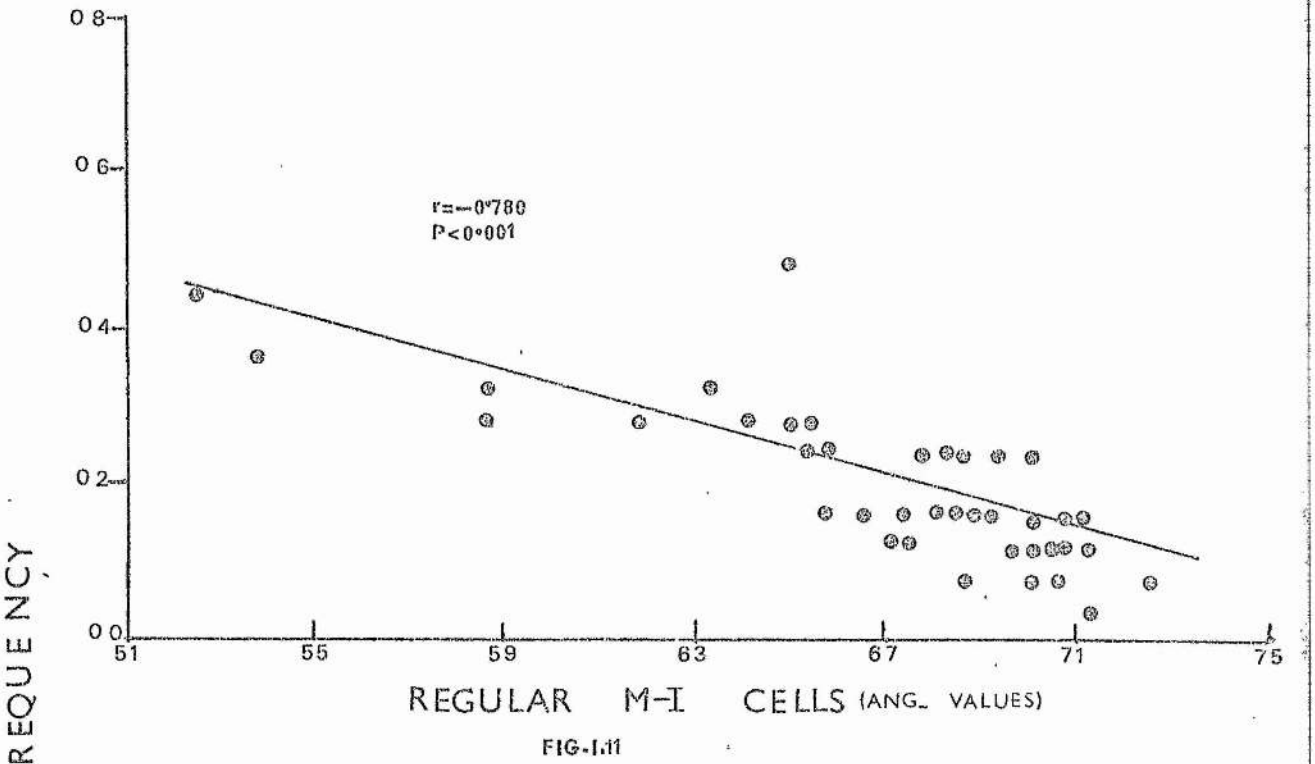


FIG-I.11

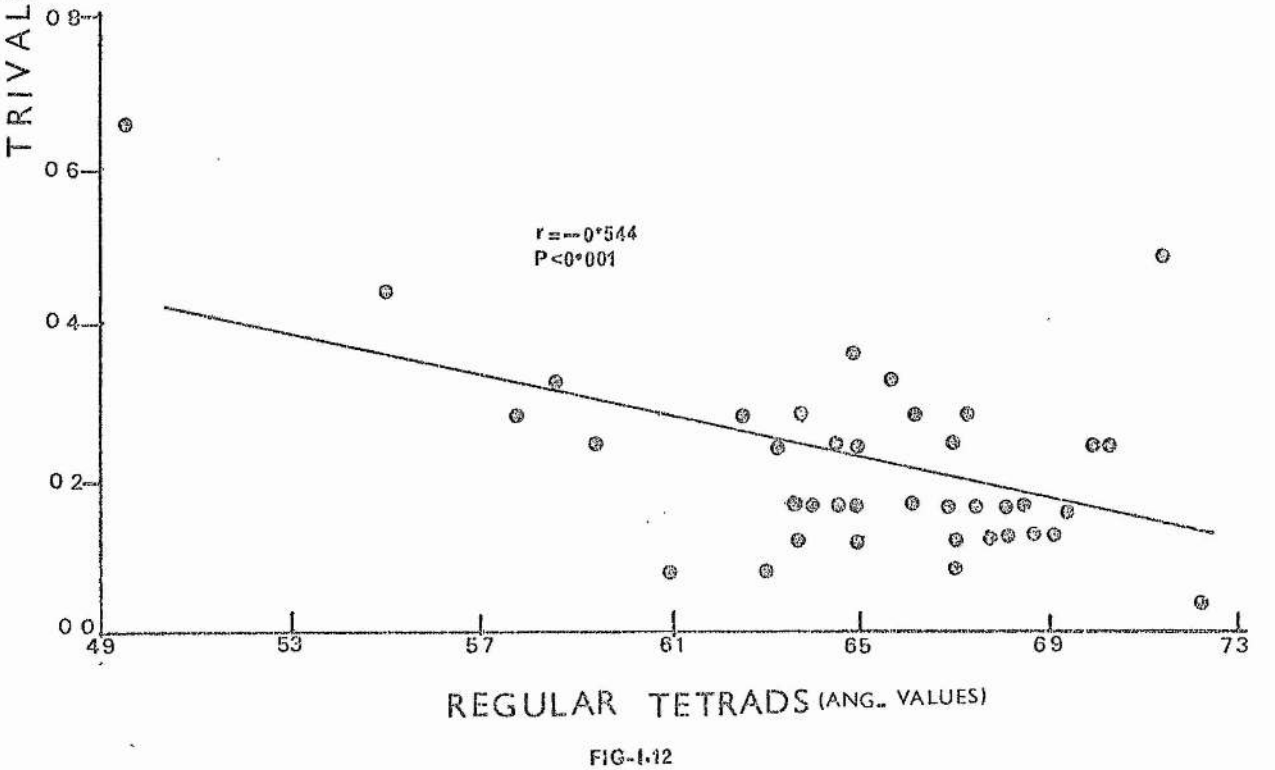


FIG-I.12

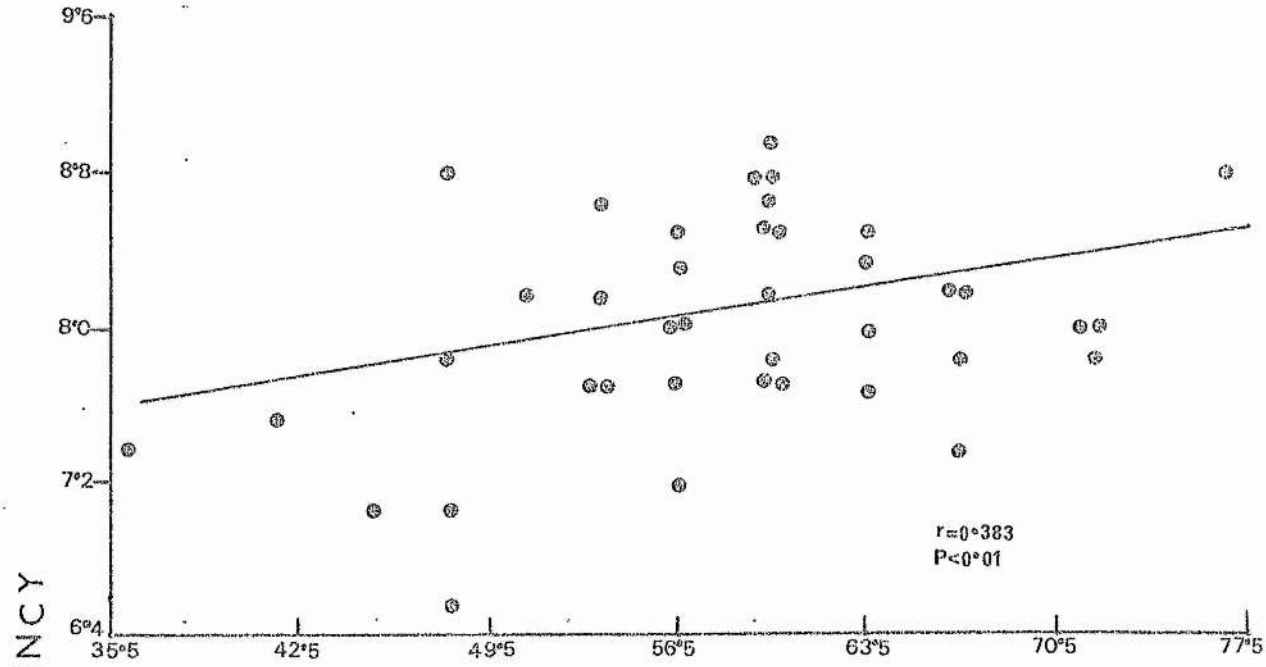


Fig. 1.13

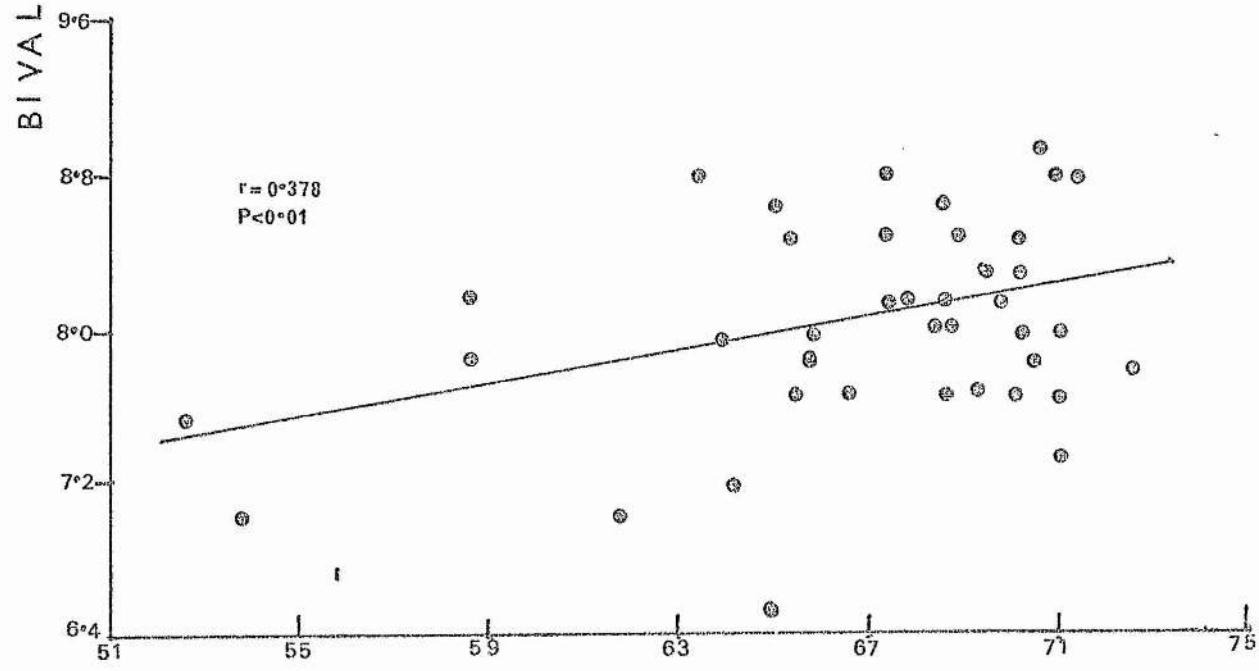


Fig. 1.14

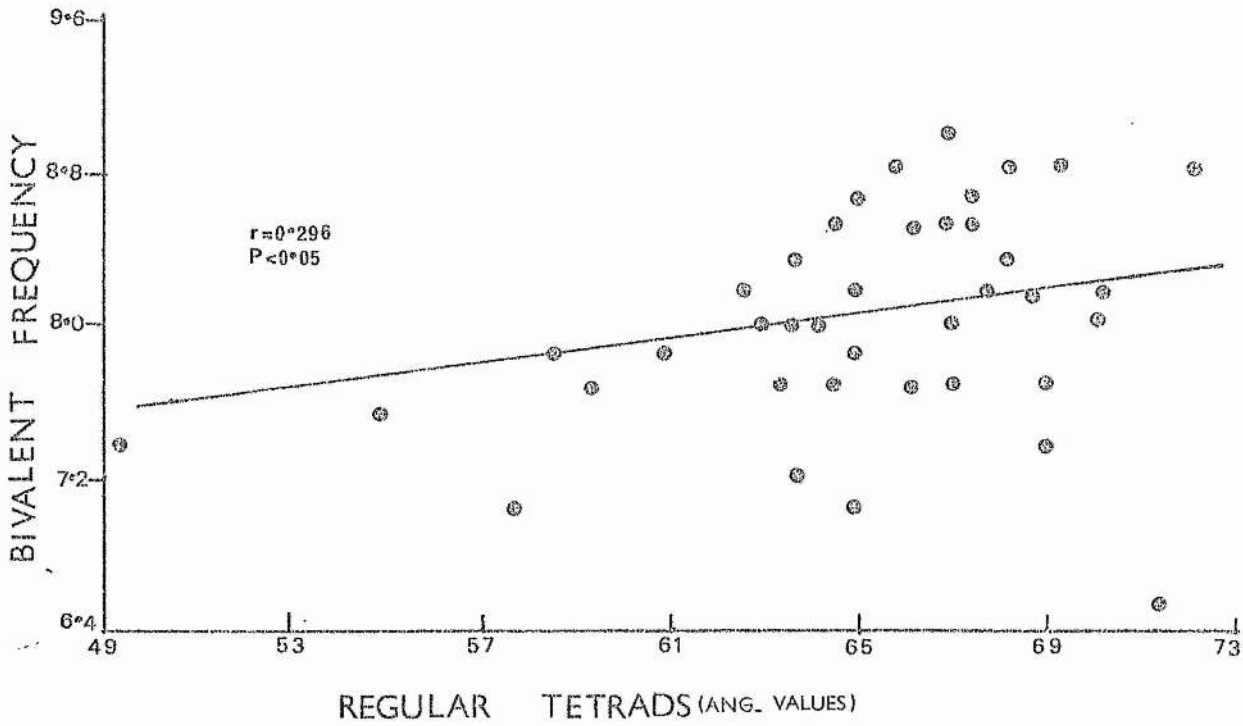


Fig. 1.15

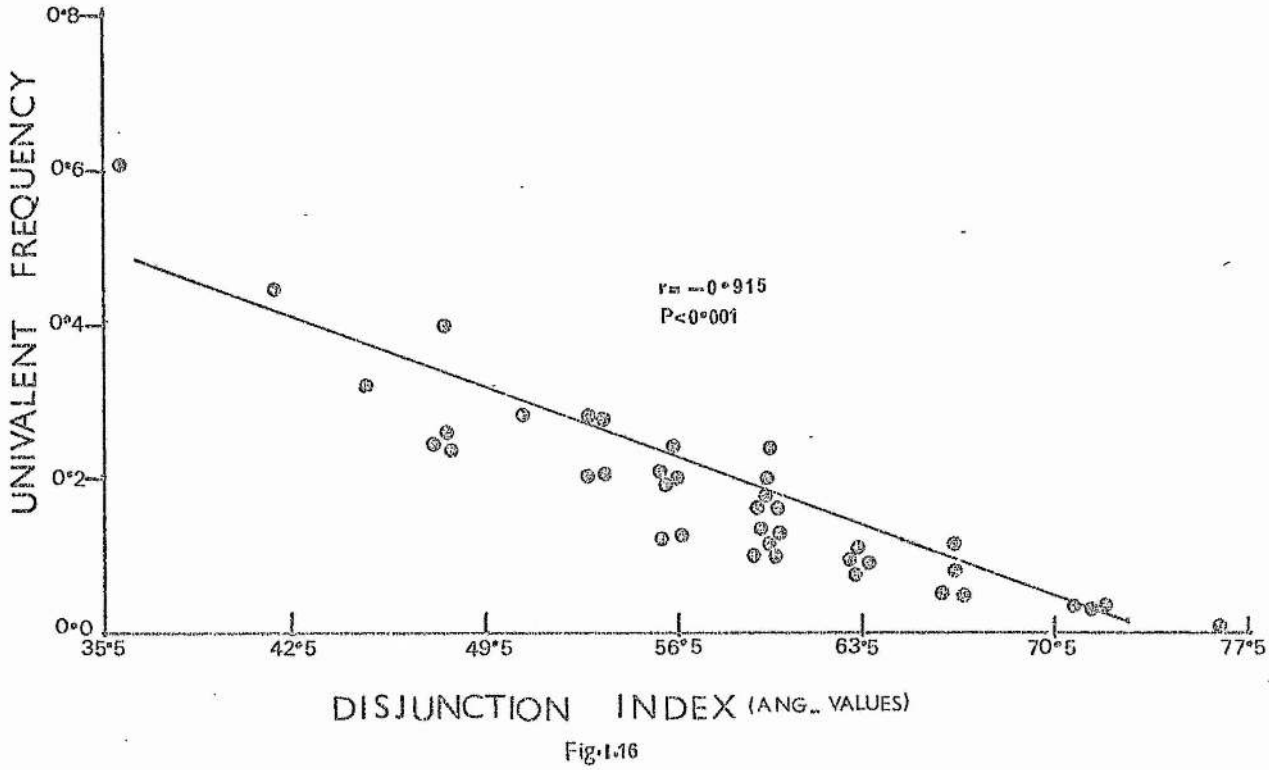


Fig. 1.16

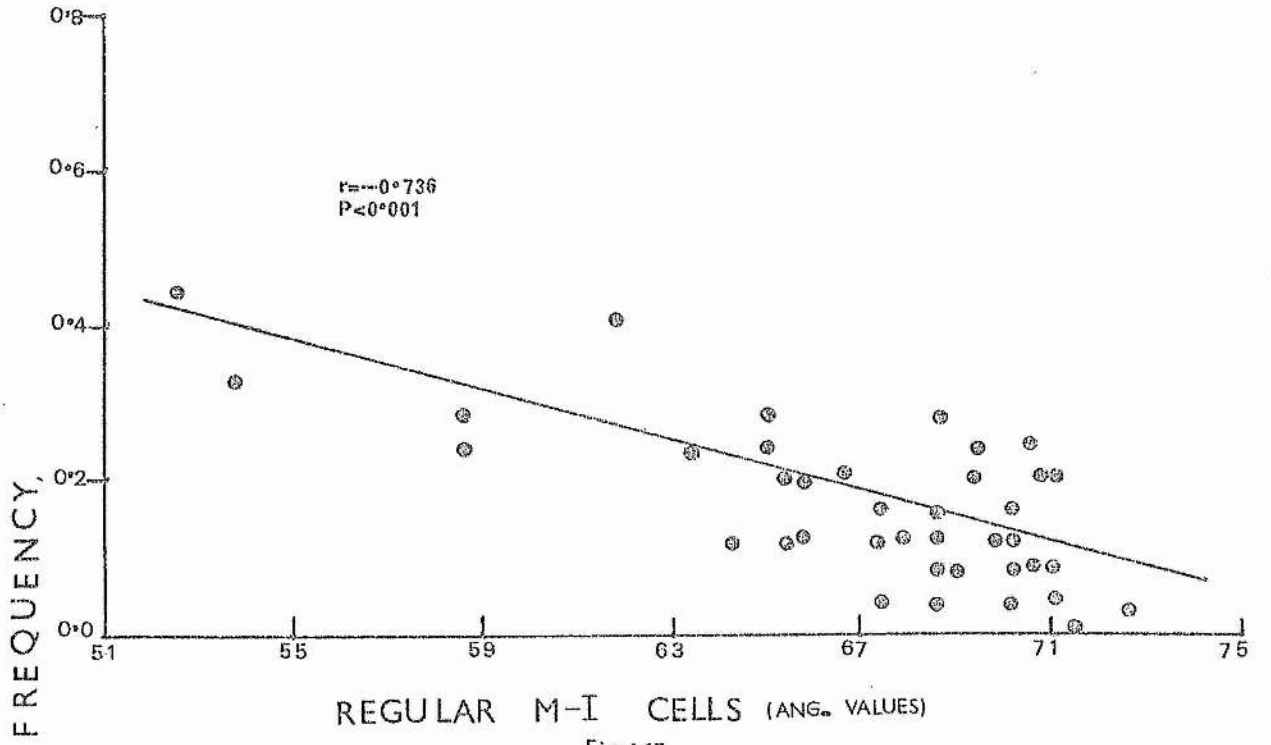


Fig. 1·17

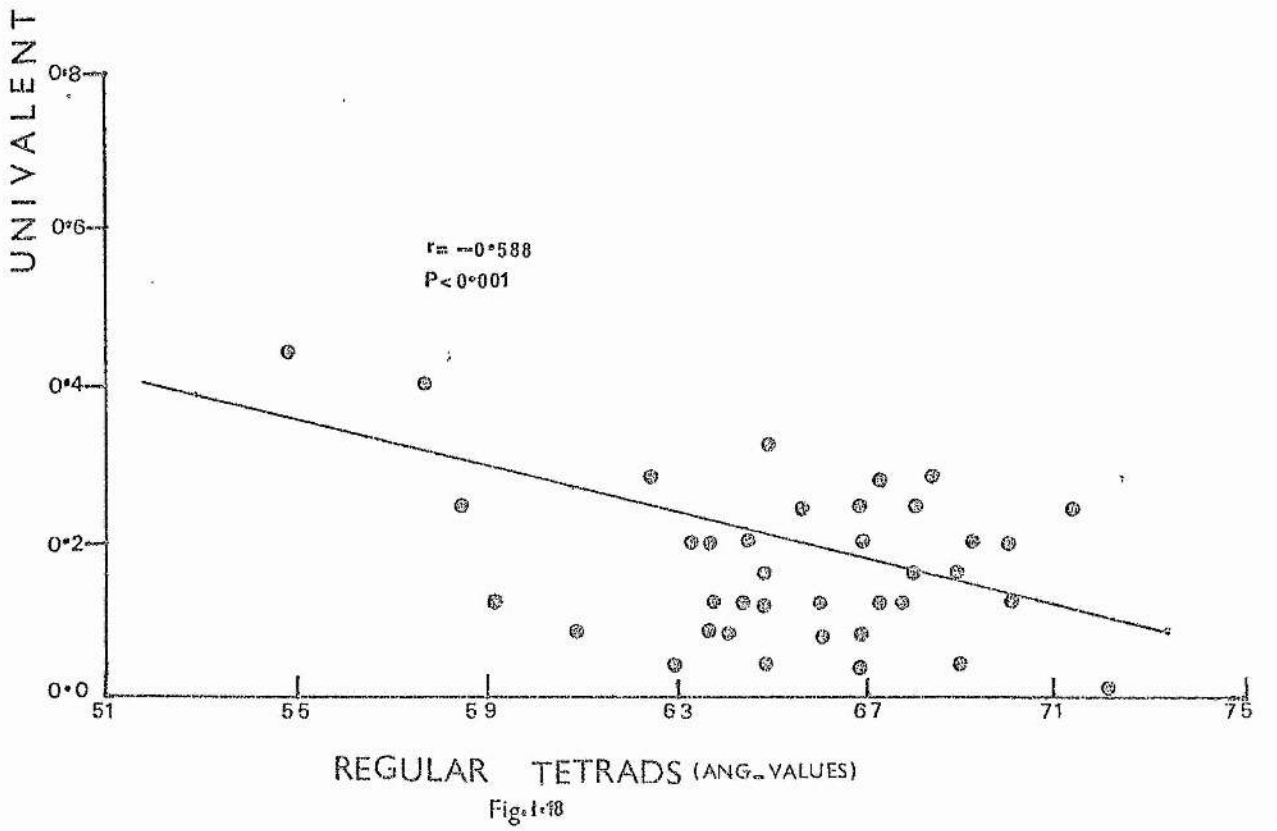


Fig. 1·18

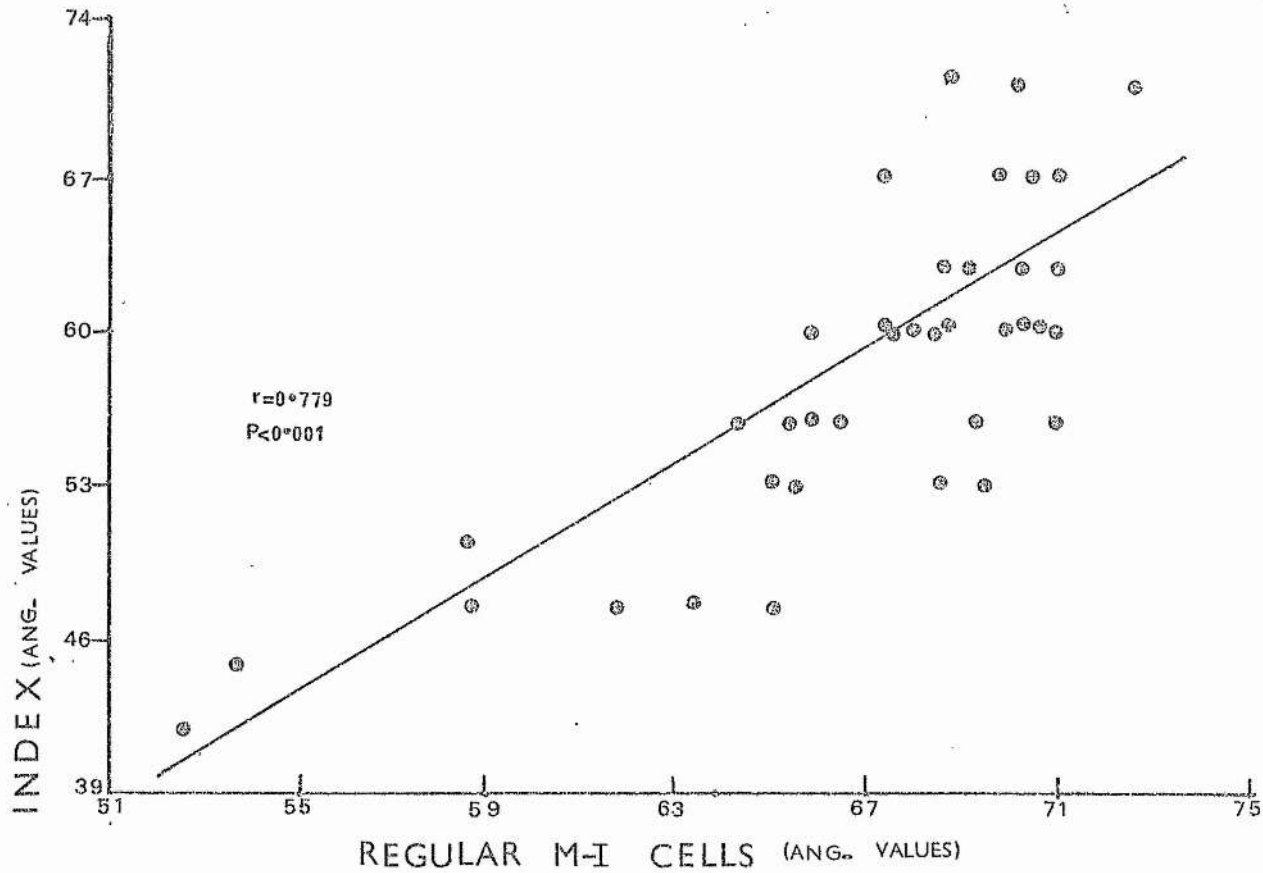


Fig. I.19

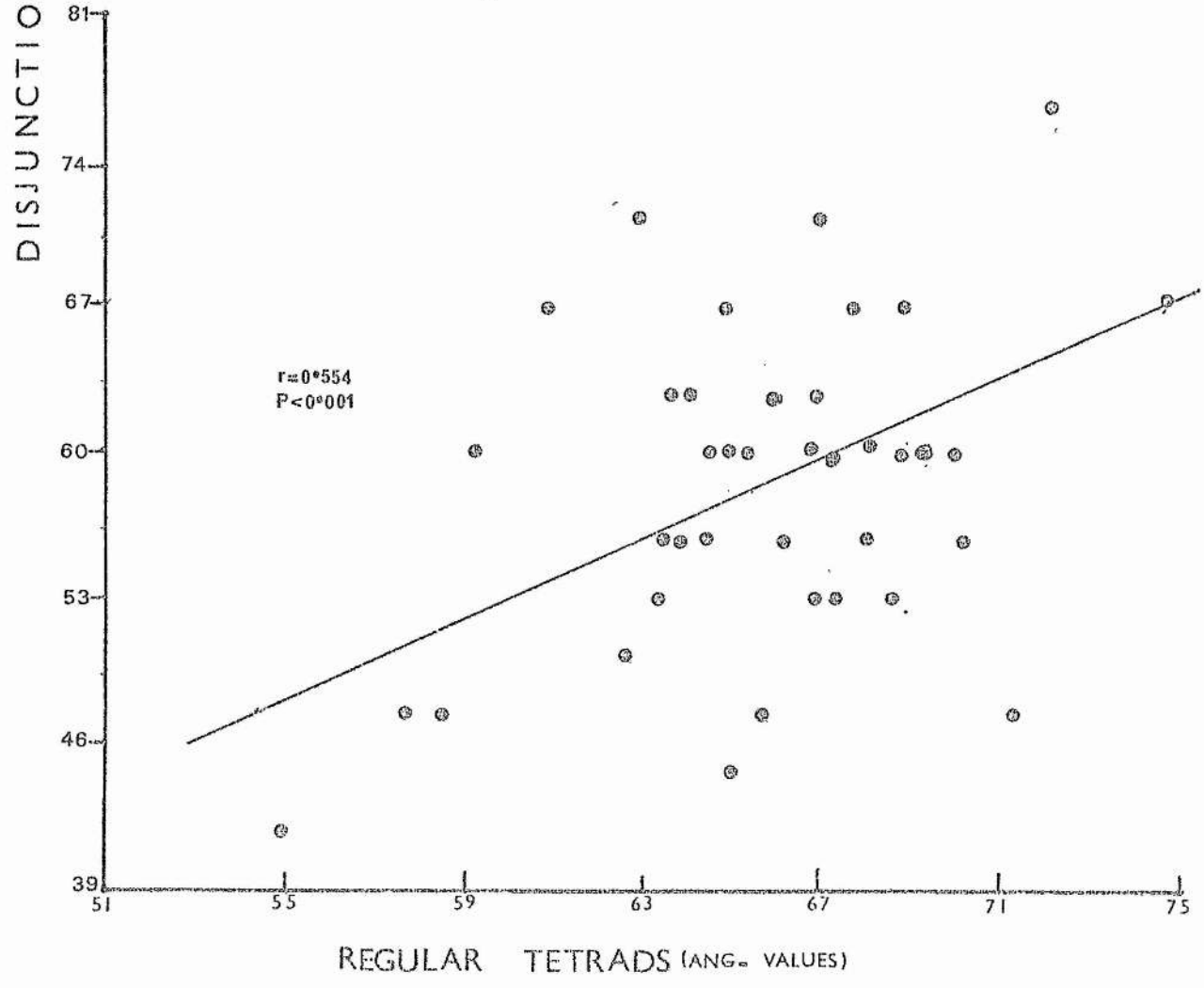


Fig. I.20

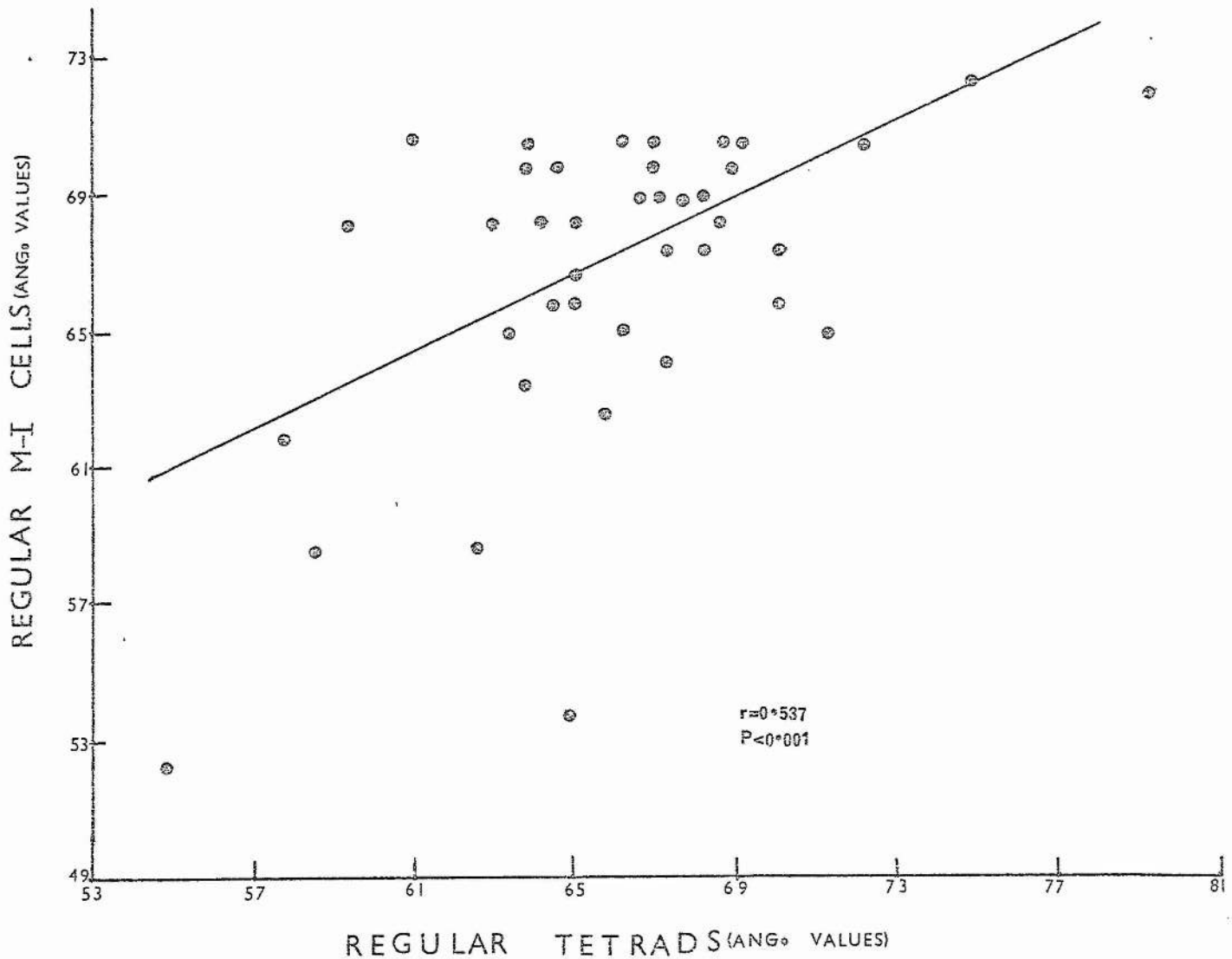

REGULAR M-I CELLS (ANG. VALUES)
73
69
65
61
57
53
49
53
57
61
65
69
73
77
81
r=0·537
P<0·001
REGULAR TETRADS (ANG. VALUES)

Fig. 1.21

Quadrivalent frequency is not correlated with any other meiotic features except having a strong negative correlation with bivalent frequency ($r_{(38)} = -0.863$, $P < 0.001$; Figure I.8). This suggests, as one would expect, bivalents are formed mainly at the expense of quadrivalents. The negative correlation between trivalents and bivalents ($r_{(38)} = -0.466$, $P<0.05$) indicates that the failure of trivalent formations also significantly contributes to bivalents. On the other hand, bivalent frequency does not show any significant relationship with univalent frequency. This suggests that in the present population there is little failure of bivalent formation which would give rise to univalents. There is, however, significant correlation of bivalent frequency with each of disjunction index (Figure I.13), regular metaphase-I cells (Figure I.14) and regular tetrads (Figure I.15). The significance of these correlations, especially of bivalents with other meiotic features, will be taken up under discussion.

The strong positive correlation between the frequencies of trivalents and univalents (Figure I.9) implies, as expected, that the occurrence of univalents is strongly associated with the occurrence of trivalents. Furthermore, both trivalent and univalent frequencies have significant and negative relationship with each of disjunction index (Figures I.10 & I.16), regular metaphase-I cells (Figures I.11 & I.17) and regular tetrads (Figures I.12 & I.18). These correlations

confirm the conclusion by Hazarika & Rees (1967) that apart from univalents, the "culprits are the trivalents" in regular chromosome separation.

III Changes in Chromosome Association Pattern from Earlier C-generations

It is of interest to know the changes that might have taken place in the meiotic behaviour since chromosome doubling about 20 years ago. As stated earlier, Hilpert (1957) and Moore (1963) studied the meiotic behaviour of the material in early C-generations. Since both workers have given data from random samples of unselected population, it was thought that a comparative study in relation to the present population would be valid in many respects. Table I.9 shows the mean number of chromosomes involved in different configurations obtained by the two earlier workers and in the present study.

Table I.9. Mean Number of Chromosomes Involved in Different Chromosome Configurations at Metaphase-I in Euploid Plants examined by Hilpert (1957), Moore (1963) and in the present study (1972).

CONFIGURATIONS	Hilpert (1957)	Moore (1963)		Present Study
	Svalöf, Sweden 1953	Svalöf, Sweden 1958	Davies, USA 1958	Newcastle, UK 1972
IVs	6.20	7.68	7.68	10.72
IIIs	1.43	1.35	1.65	0.72
IIs	18.90	18.82	18.06	16.18
Is	1.57	0.45	0.75	0.39
Total	28.10	28.30	28.14	28.01

The table shows a reduction in the number of chromosomes involved in trivalents and univalents. Parallel to this there is an increase in the number of chromosomes involved in quadrivalent formations. It is worth noting that the number of chromosomes involved in bivalents has decreased slightly. This is probably because some of the bivalents formed in early C-generations resulted from the failure of quadrivalent formations. With the improvement of meiotic stability, these chromosomes are now successfully able to form quadrivalents.

Since all the figures in the table I.9 represent data from unselected populations, it can be assumed that the improvement in meiotic stability has been achieved through natural selection. As none of the earlier workers gave data for chiasma frequency, it is not clear whether the improvement is due to an increase in chiasma frequency or due to the redistribution of chiasmata as Crowley & Rees (1968) reported for Lolium perenne. The former cause may be more likely because in "raw" autotetraploids chromosome association is impaired frequently with a reduction in chiasma number and this is further substantiated by the increased frequencies of trivalents and univalents in the early generations, the configurations which reduce the chiasma frequency. It should, however, be pointed out that the comparison ignores the presence of any significant influence of climatic and other associated factors on chromosome association.

IV. Seed-Set

In table I.10 below are presented the correlation coefficients of seed-set with different meiotic properties. None of the meiotic features show any significant relationship with seed-set. This was further confirmed by multiple regression of seed-set on three cytological features namely, (i) chiasma frequency, (ii) disjunction index and (iii) regular tetrads as independent variables. The analysis of the multiple regression is given in table I.11 and the regression is insignificant.

These results are in agreement to those reported by Muntzing (1951), Morrison (1956), Walther (1959) and Moore (1963). Most of these workers held the view that "physiological factors" are involved and their interference disturbs the correlation. On the other hand, Roseweir & Rees (1962) and Nazarika & Rees (1967) have demonstrated that chromosome pairing behaviour is in fact important in determining fertility. Their success in demonstrating the correlation between cytological features and fertility apparently lies in the fact that the variability caused by the so-called physiological factors was reduced to a minimum by long term inbreeding. As a result the influence of chromosome pairing behaviour on seed-set became evident in their experimental material.

Table I.10. Correlation Coefficients of Seed-set with Different Meiotic Properties in the Unselected Population

MEIOTIC PROPERTIES	CORRELATION COEFFICIENT	DF	P
1. Chiasma Frequency	0.273	38	n.s.
2. Cell-Variance for Chiasmata	0.044	38	n.s.
3. Quadrivalent Frequency	-0.123	38	n.s.
4. Trivalent Frequency	-0.049	38	n.s.
5. Bivalent Frequency	0.162	38	n.s.
6. Univalent Frequency	-0.153	38	n.s.
7. Number of Chromosomes in IVs + IIs	0.147	38	n.s.
8. Number of Chromosomes in IIIs + Is	-0.117	38	n.s.
9. Disjunction Index	0.093	38	n.s.
10. Regular M-I Cells	0.137	38	n.s.
11. Regular Tetrads	0.077	38	n.s.

n.s. = not significant

Table I.11. Analysis of Multiple Regression of Seed-set on Meiotic Properties (Chiasma Frequency, Disjunction Index, and Regular Tetrads)

ITEMS	D.F.	S.S.	M.S.	F	P
Regression	3	149.9818	49.9939	1.297	n.s.
Error	36	1387.4423	38.540		

n.s. = not significant

Like meiotic properties, the morphological characters also failed to show any significant correlation with seed-set (Table I.12). The lack of correlation of seed-set with both cytological features and morphological characters may be due to an interaction between the so-called physiological factors and cytological factors in such a way that when one set of factors falls short, the other set of factors comes to the aid of seed-set. This will be examined in detail in the following section (see Seed-set under Section Two).

Table I.12. Correlation Coefficients of Seed-set with Morphological Characters

MORPHOLOGICAL CHARACTERS	CORRELATION COEFFICIENT	D.F.	P
Plant Height	0.174	38	n.s.
No. of Tillers per Plant	0.005	38	n.s.
No. of Spikelets per Spike	0.005	38	n.s.
Spike Length	-0.058	38	n.s.

n.s. = not significant

DISCUSSION

One of the interesting features of the chromosome association pattern in the unselected population was the lack of correlation of quadrivalent frequency with chiasma frequency, a feature not encountered in similar studies in inbred lines (Nazarika & Rees, 1967). This was not surprising. As we know that the relative frequencies of different configurations in an autotetraploid depend not only on the number of chiasmata but also on their distribution pattern (Nazarika & Rees, 1967; Jones, 1967). The chiasma distribution pattern may affect pairing configurations at two levels. First, at the level of individual chromosome sets, depending on whether each homologous set shares an equal or unequal number of chiasmata (Jones, 1967) and, second, within the level of each set of chromosomes, depending on the presence or absence of pairing restriction (Timmis & Rees, 1971).

When there is no restriction in the association of the four homologues, one would expect a strong tendency for multivalent (quadrivalent) formation and this will be associated with increased chiasma frequency. On the other hand, if the chromosome association is restricted between "pairs" as Timmis & Rees (1971) observed in rye, bivalent formation will be predominant with increased chiasma frequency. In the former case the correlation between chiasma frequency and

quadrivalent frequency will be positive as demonstrated by Hazarika & Rees (1967) while in the latter case the correlation will be positive with bivalent frequency. In the present unselected material the absence of correlation of chiasmata with quadrivalents and the presence of significant and positive correlation with bivalents suggest that a restriction in chromosome association between "pairs" is pronounced in the unselected material.

It is not unlikely that the unselected population after about 15 generations of random mating may have reached an "equilibrium state" with respect to its pairing behaviour. The absence of correlations of cell variance for chiasmata with other meiotic features (see Table I.8) and the insignificant differences between the plants with respect to quadrivalent, bivalent and trivalent frequencies may be indicative of such an "equilibrium state".

Having indicated the existence of pairing restriction and equilibrium state in the material, it would be desirable to see if the changes in pairing pattern can be caused by a change in the breeding system. Evidence given by Ellis et al. (1973) shows that in artificial autotetraploid Poa annua which predominantly forms bivalents, a significant increase in quadrivalent frequency can be caused by enforced selfing. This is presumably caused by the approach to homozygosity with accompanying increases in homology between the four

representative chromosomes in the selfed progenies. This suggests that in outbreeding species restriction in chromosome association in "pairs" can be reduced by inbreeding. An evidence of such changes in rye can be obtained by a comparison of the number of chromosomes involved in different configurations in inbred and outbred materials. Table I.13 shows such a comparison between inbred lines used by Hazarika & Rees (1967) and the present outbred material.

Table I.13. Mean Number of Chromosomes Involved in Different Pairing Configurations and Number of Chiasmata per PMC in Inbred Lines Used by Hazarika & Rees (1967) and the Present Outbred Material.

MATERIALS	NUMBER OF CHROMOSOMES INVOLVED IN :					Total no.of chromo-somes	Average Chiasma Frequency
	IV	III	II	I	IV+III		
Inbred Lines (Hazarika & Rees, 1967)							
P1	14.48 (51.79%)	1.14 (4.08%)	11.66 (41.70%)	0.68 (2.43%)	15.62 (55.87%)	27.96	22.49
P8	12.48 (44.76%)	1.32 (4.74%)	13.38 (47.99%)	0.70 (2.51%)	14.80 (53.09%)	27.88	21.67
P12	13.76 (49.28%)	0.66 (2.36%)	12.94 (46.35%)	0.56 (2.01%)	14.42 (51.65%)	27.92	22.57
P13	15.84 (56.57%)	0.51 (1.82%)	11.40 (40.71%)	0.25 (0.89%)	16.35 (58.39%)	28.00	24.75
Outbred Material (Present Investi-gation)	10.72 (38.27%)	0.72 (2.57%)	16.18 (57.76%)	0.39 (1.39%)	11.44 (40.84%)	28.01	25.04

The table clearly shows that there are considerable differences between the outbred and the inbred materials in the number of chromosomes involved in a specific type of configurations. Of particular interest, the number of chromosomes involved in multivalent formations is considerably higher in each of the inbred lines while the number of chromosomes involved in bivalent formation is similarly higher in the outbred material. These differences are possible in the light of "free" and "restricted" pairing patterns in inbred and outbred materials respectively. It should be pointed out that ideally a comparison should be made between inbred and outbred materials of similar genetic background and origin. The above comparison nevertheless throws light on the differences in pairing pattern one may expect following a change in the breeding system. Furthermore, it lends support to the findings by Ellis, et al. (1973) that selfing increases multivalent formations.

With a pronounced "pairing restriction", disomic association (bivalent) would dominate the pairing pattern instead of quadrivalents. As a result we would expect correlations of bivalents, instead of quadrivalents, with other meiotic properties. This was in fact demonstrated in this material.

One other point which deserves a comment is the lack of correlation between bivalent frequency and the frequency of

univalents. This indicates that once the pairing between "two" homologous chromosomes has been initiated, the process continues much more efficiently than when more than two chromosomes are involved in a pairing process. In other words, bivalent formation is more easily accomplished than a multivalent formation. This will be demonstrated later where it will be shown that multivalent formation is impaired more easily than bivalents by changes in environmental agents.

To conclude, it would appear that an improvement in the meiotic behaviour could be achieved by selecting plants for increased bivalent frequency. Rye, being an outbreeding species, offers better prospects for increasing bivalent frequency by imposing pairing restriction, possibly by crossing between selected "alien" lines. Some encouraging results in this respect have been reported by Sybenga (1969 & 1973), Tarkowski (1970) and Otlowaska (1971). Until now, however, this remains a theoretical proposition, because no remarkable practical results have been achieved yet.

The absence of correlation between seed-set and any of the meiotic properties or morphological characters in the population is explained as due to the interaction of the cytological factors with the so-called physiological factors in a supplementary way in determining seed-set. This will be examined later (see Seed-set and Meiotic Features in Section Two).

S E C T I O N T W O

EFFECTS OF SELECTION FOR SEED-SET AND MEIOTIC BEHAVIOUR

INTRODUCTION

It is a well established fact that autopolyploid plants in general produce reduced numbers of seeds per plant. This reduction is largely accounted for by the decreased number of fertile flowers per plant. As stated earlier, tetraploid rye is no exception in this respect. As a result many attempts have been made to find the causes for this reduction in fertility and explore the possibilities of increasing the seed-yield. These attempts mainly include studies on meiosis behaviour in relation to seed-set and the methods of approach have been both direct and indirect.

The direct selection approach was made by Plarre (1954) and Hilpert (1957) in which they attempted selection for meiotic regularity with a view to improving the seed-set. Their bases of selection were, however, widely different. Plarre (1954) regarded all the PMCs with multivalents and univalents at metaphase-I as the 'aberrant' types and those with only ring and rod bivalents as the 'normal' types. Plants with higher frequencies of 'normal' configurations were selected and crossed. In the F_1 generation the seed-set increased by 2 per cent over the parents and 8 per cent over the original population.

Hilpert (1957) classified bivalents and zig-zag quadrivalents as "regular" configurations and chain plus ring quadrivalents, trivalents and univalents as "irregular" pairing configurations. The materials studied were accordingly divided into three groups, namely, (A) regular, (B) intermediate, and (C) irregular. The groups were kept isolated during the flowering time and meiosis was studied in the progenies. No significant differences were found between the groups with respect to different configurations. She, however, claimed that the groups A and B showed a tendency to form higher frequencies of bivalents and zig-zag quadrivalents as compared to the group C. Thus she concluded, "a single separate selection for regular meiosis has given no effect although there is a tendency in this direction".

Several other workers made indirect approaches by selecting plants for high seed-set and examining the consequent changes in meiotic regularity. Data on the effect of repeated selection for high seed-set on meiotic pairing behaviour were given by Muntzing (1951), Morrison (1956), Bremer & Bremer-Reinders (1954), Hilpert (1957) and Walther (1959).

Muntzing (1951) compared the meiotic behaviour of tetraploid Steel rye which had been rigidly selected for seven generations and three other tetraploid strains of different origin. He observed no difference between the strains in the frequencies of quadrivalents and concluded that selection for seed-set over

a period of seven years had "little or no effect" on pairing behaviour.

Morrison (1956) found no difference in chromosome association pattern between two strains of Tetra Petkus rye nor between his results and Muntzing's (1951), although the stocks belonged to different "C"-generations. This led him to conclude that any increase in seed-set must have a genetical basis or some obscure physiological cause.

Bremer & Bremer-Reinders (1954) selected the most fertile plants for seven successive generations and observed an increase in seed-set from 60% to about 75%. This was accompanied by increased regularity in meiotic divisions as observed by the number of laggards at anaphase-I and micro-nuclei in tetrads and a considerable decrease in the aneuploid frequencies in the selected material.

Hilpert (1957) claims that repeated selection for seed-set and tillering capacity for three generations resulted a significant increase in bivalent frequency accompanied by fewer quadrivalent formations.

Walther (1959) compared his meiosis data with those obtained by Flarre (1954) about five generations earlier on the same strain. The comparison seems to indicate more regular meiosis as a result of repeated selection for high seed-set.

According to these reports there is no clear indication as to the difference in meiosis behaviour between tetraploid populations. On the other hand, it has been clearly demonstrated that there is a considerable variation in the degree of meiotic irregularities within a population of tetraploid rye. Whether the seed-set within a population is related to the within population variation in meiotic regularity has been examined by several authors.

Walther (1959) and Moore (1963) examined the relationship between meiotic regularity and seed-set within two separate populations of tetraploid rye. Both of these studies failed to demonstrate a correlation. Roseweir & Rees (1962) and Hazarika & Rees (1967) from their investigations on inbred rye have shown that the frequencies of various chromosome configurations are correlated with chiasma frequency, the latter being genotypically controlled. They have shown that the seed-set in tetraploid inbred rye is positively correlated with quadrivalent frequency. But based on outbred population means Aastveit (1968) observed a correlation in the opposite direction. He reported that high seed-set was associated with lower frequency of multivalents and higher frequency of bivalents.

The various studies on meiosis and its relation with seed-set have thus yielded widely different and in many cases conflicting results. This may, in part, be due, as stated earlier, to the selective advancement of the experimental

material and in part, due to the physiological factors as pointed out by Morrison (1956), Walther (1959) and Moore (1963).

In view of the complexity of the relationship between seed-set and its causal factors, an attempt was made to select plants for seed-set and meiosis behaviour simultaneously. By such an attempt, it was expected, most of the factors influencing fertility, whether these are cytological, physiological or genetical, could be taken into account.

MATERIALS AND METHODS

The material of tetraploid rye used in the investigation was obtained from the selection material of Dr. K. Moore. The following information as to the origin of the material was kindly furnished by him.

'Seeds from randomly selected plants of "Fourex" rye (2n = 4x = 28) used by Moore (1963) were sown in 1967. Young spikes from fifty plants were fixed in acetic alcohol (1:3) for meiosis studies. One spike of each fixed plant was selfed by wax-paper bagging before anthesis. Euploid plants were selected in two groups:

(1) High seed-set: With more than 70% seed-set and regular meiosis (above 82.5% regular tetrads)

(2) Low seed-set: With less than 60% seed-set and irregular meiosis (less than 80% regular tetrads).

In 1968 seeds from the selected plants were grown. The two groups were grown in isolation. The selfed seeds from each parental plant represented a line. Young spikes from several plants within a line were fixed for meiosis studies and the fixed plants within a line were crossed. Euploid plants were again selected for "high" seed-set and "low" seed-set as above. Since it was not possible to undertake meiosis

studies in all the plants, the crossed seeds were selected mainly on the basis of parental seed-set. The selected seeds from each cross were sown in individual lines in 1970 and again the "high" seed-set material was grown in isolation from the "low" seed-set material.'

The materials were handed over to me at this stage in 1970.

Handling of the Materials and Morphological Observations:

A single spike from a plant was fixed in acetic alcohol (1:3) for meiotic studies. Several plants within a line were fixed and they were numbered (1, 2, etc.) in accordance with the line identity. Before anthesis the fixed plants within a line were crossed in pairs by enclosing the two selected spikes in a paper-bag. The other spikes of the plant were left to set seed by outcrossing. Prior to harvesting the crossed seeds were collected. The following observations were made from the plants in each line:

(1) Plant height (cms.)

(2) Number of tillers per plant

(3) Number of spikelets per spike

(4) Spike length (cms.)

(5) Seed-set (%)

Except observation (2) above, all other characters were recorded from the tallest tiller of the plant.

Selection of crossed seeds were made from euploid parents. In the "high" seed-set group those crossed seeds were selected where both parents had above 70% seed-set and more than 80% regular tetrads (i.e. tetrads without micronuclei, anaphase-II bridges and polyads). While in the "low" group the basis of selection was less than 65% seed-set and less than 80% regular tetrads in the parents.

In 1971 the lines of "high" and "low" groups were sown in alternate rows. The plants were fixed, crossed and selected in the same way as with 1970 materials. The observations made were also similar to those in 1970.

In 1972 a comparison trial, comprising (i) "high", (ii) "low" and (iii) unselected materials of the same C-generation, was conducted. The lines from the three groups were sown in randomised order in an attempt to reduce the effect, if any, of pollen from adjacent rows on seed-setting. As many plants as possible were fixed from each line and fixing continued from late-May to mid-July, 1972.

Cytological Methods and Observations:

In the fixed materials of 1970 no serious attempt was made for detailed metaphase-I analysis. Cytological observations were concentrated on the frequencies of regular metaphase-I cells (i.e. PMCs without univalents) and regular tetrads

(i.e. tetrads without micronuclei, anaphase-II bridges and polyads). In 1971 fixed materials, meiotic studies could not be completed before the following sowing time. Therefore, the plants grown in 1972 were from crossed seeds selected on the basis of parental seed-set only. However, detailed meiotic studies were undertaken in the three plant populations grown in 1972. The cytological methods followed and the observations made have been described under MATERIALS AND METHODS in SECTION ONE.

RESULTS

1. High and Low Seed-set Populations, 1970

A. The Means

The means and the standard errors of five morphological characters and two meiotic features of euploid plants of "high" and "low" populations are presented in table II.1. In the same table the results of comparison between the two populations are given. The comparisons were made according to t-test for unequal sample size and separate variance estimate (Nie & Hull, 1973). The table shows that the two populations differ significantly in all the morphological characters except number of spikelets per spike. The two meiotic features, however, did not show any significant differences between the two populations.

Since the plants were deliberately selected for seed-set in the two populations, the difference observed in this character (i.e. seed-set) was expected. Associated with this, reductions in vegetative performances such as plant height, number of tillers in the "low" populations indicate a general poor vigour of plants in this population. Whether the poor vigour in the "low" population reflects the effects of selection could not be ascertained because it was found out that the "high" population received an additional fertilizer treatment

(approx. 68.88 lbs N, 70.56 lbs K_2O & 56.95 lbs P_2O_5 per acre) which presumably contributed to the more vigorous growth of plants compared to those in the "low" group.

Table II.1. Five Morphological Characters and Two Meiotic Features of Euploid Plants from "High" and "Low" Populations, 1970

CHARACTERS	MEANS		D.F. for separate variance estimate	t-value for separate variance estimate	Probability
	HIGH	LOW			
Morphological Characters	n = 113	n = 71			
Seed-set (Angular Values)	62.12±0.597	52.97±0.953	123.89	8.14	0.001
Plant height (cms)	135.04±0.998	128.20±1.273	147.66	4.23	0.001
No. of tillers per plant	6.98±0.299	4.34±0.260	180.34	6.68	0.001
No. of spikelets per spike	25.19±0.277	25.04±0.385	138.35	0.30	n.s.
Spike length (cms)	10.36±0.111	9.82±0.156	136.71	2.83	0.01
Meiotic Features	n = 79	n = 47			
Regular M-I Cells (Angular Values)	63.82±0.547	63.85±0.600	109.54	0.03	n.s.
Regular Tetrads (Angular Values)	64.20±0.537	65.24±0.696	96.75	1.18	n.s.

B. Seed-set in Relation to Other Characters (1970)

Correlation coefficients of seed-set with four vegetative characters and two meiotic features in "high" and "low" populations are presented in table II.2. The table also gives the heterogeneity tests for the correlation coefficients in the two population.

Table II.2. Correlation Coefficients of Seed-set with Four Vegetative Characters and Two Meiotic Features in "High" and "Low" Populations, 1970

CHARACTERS	HIGH	LOW	Heterogeneity of Correlation Coefficients		
Vegetative Characters	n = 113	n = 71	Chi-square	D.F.	Probability
Plant height	0.160*	0.142	1.322	1	0.20 -0.30
No. of tillers per plant	0.022	0.005	-	-	-
No. of spikelets per spike	0.169*	0.147	0.019	1	0.80 - 0.90
Spike length	0.178*	0.225*	0.118	1	0.70 -0.80
Meiotic Features	n = 79	n = 41			
Regular M-I Cells	0.165	0.228	0.091	1	0.70 -0.80
Regular tetrads	0.125	0.181	0.066	1	0.70 -0.80

* indicates significant at 5% level.

While in the "high" population seed-set is significantly correlated with plant height, number of spikelets per spike and spike length, the correlation in the "low" population is significant only with spike length. None of the two meiotic features has any significant relationship with seed-set (see table II.2).

As mentioned above, the "high" population received an additional fertiliser treatment and those plants which were capable of utilising the added nutrients were presumably more fertile. This might have contributed to slightly higher correlation of seed-set with morphological characters in the "high" population.

This can be critically examined by heterogeneity tests for the corresponding pairs of correlation coefficients. One may be tempted to claim from the differences in means (Table II.1) and the significant correlation (Table II.2) in the high population that the selection has been effective at this stage. If this is so, one would expect significant heterogeneity of correlation coefficients between the two populations because the populations should be genetically different with successful selection. On the other hand, samples from genetically identical populations would not show any significant heterogeneity in the correlation coefficients. The heterogeneity tests for correlation coefficients appear in table II.2. We do not find any evidence of heterogeneity in any characters.

It can, therefore, be inferred that the two populations, "high" and "low", represent samples of genetically similar populations. In other words, there is not sufficient evidence to claim that the selection has yet produced two populations which can be regarded to have diverged genetically.

It is, therefore, apparent that the additional fertiliser treatment in the "high" population increased its mean seed-set and also was responsible for the significant correlation between vegetative characters and seed-set. This indicates the possible relationship between 'physiological vigour' and seed-set of a plant. The effects of nitrogenous fertiliser on seed-fertility in autotetraploid rye has already been reported by Ellerström & Sjodin (1963). They suggested that the increased seed-set in such cases is mainly due to the favourable conditions for seed-development provided for by the added nutrients and not attributable to meiotic behaviour. The latter can be envisaged from the insignificant differences in the two meiotic characters in the "high" and the "low" populations.

2. High and Low Seed-set Selected Samples, 1971

Since the chromosome number of individual plants were not determined and selection was based only on seed-set, it is probable that the selected samples in both groups included aneuploid individuals and the frequency of aneuploids for obvious reasons was likely to be higher in the "low" seed-set sample. The comparison presented in table II.3, therefore, ignores the frequency of aneuploids.

The table shows that the two samples differed significantly only with respect to seed-set ($P<0.001$) and plant height ($P<0.001$). These differences might have been due to the higher frequency of aneuploids in the "low" seed-set sample.

Table II.4 gives the correlation coefficients of seed-set with morphological characters. Only a single correlation is significant in the "high" seed-set sample whereas in the "low" sample the correlation is significant with plant height ($P<0.05$), spikelet number and spike length ($P<0.01$). Again this is presumably due to a higher frequency of aneuploids in the "low" sample, because aneuploids are normally less fertile with poor vigour. The heterogeneity tests for the correlation coefficients reveal that only the correlations with respect to plant height differ significantly between the two samples. Since the two populations were grown under similar conditions in the field,

Table II.3. Comparisons of Morphological Characters between Selected High and Low Seed-set Samples, 1971

CHARACTERS	MEANS		D.F. for separate variance estimate	t-value for separate variance estimate	Probability
	HIGH	LOW			
	n = 45	n = 30			
Seed-set (Angular Values)	64.58±0.873	48.02±1.468	49.09	9.70	0.001
Plant height (cms)	144.73±2.045	134.57±3.279	50.88	2.63	0.01
No. of tillers per plant	4.58±0.230	3.97±0.327	55.85	1.53	n.s.
No. of spikelets per spike	27.87±0.487	26.83±0.567	64.40	1.38	n.s.
Spike length (cms)	10.79±0.204	10.80±0.237	64.53	0.04	n.s.

n.s. = not significant

Table II.4. Correlation Coefficients of Seed-set with Vegetative Characters

CHARACTERS	HIGH	LOW	Heterogeneity Tests for Correlation Coefficients		
	n = 45	n = 30	Chi-square	D.F.	Probability
Plant height	0.196	0.420*	5.515	1	0.01 - 0.02
No. of tillers per plant	-0.251	0.031	-	-	-
No. of spikelets per spike	0.304*	0.365*	0.081	1	0.70 - 0.80
Spike length	0.270	0.470**	0.142	1	0.70 - 0.80

n.s. = not significant

* = significant at 5% level

** = significant at 1% level

the above heterogeneity may either indicate genetic difference between the two populations or this may be solely due to the higher aneuploid frequency in the "low" sample. Here too one cannot, therefore, be certain about the effects of selection. However, a detailed comparison between the progenies of these two selected samples and the unselected population was made in the following year (1972) and the results of the trial are shown in the next chapter.

3. High, Low and Unselected Populations, 1972

A. Random Samples

I. Frequencies of euploid, aneuploid and structurally aberrant plants in the three populations

In 1972 plants of "high", "low" and unselected populations were grown in the field in randomly arranged rows. As many plants as possible were fixed from each group for cytological observations. Table II.5 gives the frequencies of euploids, aneuploids and structurally aberrant plants in the three populations.

Table II.5. Frequencies of Euploids, Aneuploids and Structurally Aberrant Plants in High, Low and Unselected Populations, 1972.

POPULATIONS	No. of Plants with Chromosome Number						No. of Plants with Structural Aberrations		Total
	26	27	28	29	30	31	Translocation Heterozygote	Inversion Heterozygote	
High	-	16 6.75%	199(1) 83.97%	18 7.59%	3 1.27%	-	-	1 0.42%	237
Low	1 0.81%	8 6.50%	85 69.11%	23(1) 18.70%	1 0.81%	1 0.81%	4 3.25%		123
Unselected	-	5 4.39%	86 75.44%	18 15.79%	2 1.75%	-	3(2) 2.63%	-	114

(1) Includes one plant with 27 + one centric fragment

(2) Includes one 29 chromosome plant

There are approximately 16%, 31% and 25% aneuploids plus cytologically aberrant plants in the "high", the "low" and the unselected populations respectively. It is also worthwhile to note that the aneuploid frequency in the "high" population is considerably less not only than in the "low", but also the unselected population. This illustrates one beneficial aspect of selection.

II. The Sample Means

Besides any genetical differences between populations, the varied frequencies of aneuploids will affect the average seed-set and vegetative characters of the respective populations. Table II.6a gives the sample means for various characters in the three populations while table II.6b gives the results of comparisons between populations.

It will be seen that the mean seed-set of the high population does not differ significantly from that of the unselected population whereas the mean of the low population is significantly less than either of the former two. In relation to the frequencies of aneuploids, these results are interesting. While the aneuploid frequency in the unselected population is higher by about 10% than that in the high population, in contrast to only 6% difference between the unselected and the low, the former two populations (high and

Table II.6a. Means for Seed-set and Morphological Characters in High, Low and Unselected Populations (including aneuploids and cytologically aberrant plans), 1972

CHARACTERS	HIGH	LOW	UNSELECTED
	n = 232	n = 122	n = 108
Seed-set (Angular Values)	53.63±0.726	48.72±0.989	54.50±0.864
Plant height (cms)	124.07±1.271	119.86±1.946	133.09±1.808
No. of tillers per plant	5.82±1.271	6.28±0.303	6.42±0.321
No. of spikelets per spike	26.00±0.265	25.71±0.385	26.05±0.401
Spike length (cms)	10.61±0.117	10.60±0.176	11.07±0.175

Table II.6b. Comparisons of High, Low and Unselected Populations, 1972

CHARACTERS	POPULATIONS COMPARED	D.F. for separate variance estimate	t-value for separate variance estimate	Probability
Seed-set	(a) High/Low	248.79	4.00	<0.001
	(b) High/Unselected	253.19	0.77	n.s.
	(c) Low/Unselected	226.78	4.40	<0.001
Plant height	(a) High/Low	224.86	1.81	n.s.
	(b) High/Unselected	214.72	4.08	< 0.001
	(c) Low/Unselected	227.97	4.98	< 0.001
No. of tillers per spike	(a) High/Low	218.73	1.29	n.s.
	(b) High/Unselected	185.96	1.61	n.s.
	(c) Low/Unselected	224.84	0.31	n.s.
No. of spikelets per spike	(a) High/Low	235.00	0.64	n.s.
	(b) High/Unselected	202.80	0.09	n.s.
	(c) Low/Unselected	225.64	0.61	n.s.
Spike length	(a) High/Low	228.91	0.06	n.s.
	(b) High/Unselected	205.53	2.16	<0.05
	(c) Low/Unselected	227.27	1.88	n.s.(> 0.05)

unselected) do not differ significantly with respect to seed-set whereas the difference between the latter two (low and unselected) is highly significant ($P < 0.001$). This suggests that aneuploid frequency cannot always account for the total differences in fertility between populations. Genetical constitution including the degree of heterosis and physiological factors would also influence the average seed-set of a population. While the two selected groups, "high" and "low", have been partially inbred for several generations, the unselected materials represent a natural population, evidently with a higher degree of heterosis. This apparently helped the unselected population to overcome the effect of/higher frequency of aneuploids compared to the "high" population. On the other hand, with a higher aneuploid frequency in the "low" population the mean seed-set is considerably reduced. This may, in part, be due to the genetical constitution of the "low" population which has been selected for reduced seed-set over four generations.

III. Seed-set in Relation to Morphological Characters in the Three Populations

The correlation coefficients of seed-set with morphological characters in the random samples of "high", "low" and unselected populations are presented in table II.7. Each of the four

morphological characters is significantly correlated in the "high" population. Similarly in the "low" and the unselected populations the correlations are significant with the exception of number of tillers per plant. Moore (1963) reported similar results for samples including aneuploids where plant height was significantly correlated with seed-set. He further demonstrated that when only euploids were considered, the correlation became insignificant. Evidently this is due to the reduced fertility corresponding with poor vigour of the aneuploids compared to the euploid plants. The same holds true for other morphological characters.

Table II.7. Correlation Coefficients of Seed-Set with Morphological Characters in Random Samples from High, Low and Unselected Populations, 1972.

CHARACTERS	Correlation Coefficients			Heterogeneity Test for Correlation Coefficients		
	High n = 232	Low n = 122	Unselected n = 108	Chi-square	D.F.	P
Plant height	0.488***	0.434***	0.578***	25.669	2	0.001
No. of tillers per plant	0.205**	0.132	0.097	0.979	2	0.50-0.70
No. of spikelets per spike	0.205**	0.279**	0.359***	0.580	2	0.70-0.80
Spike length	0.219***	0.245**	0.321***	1.491	2	0.30-0.50

Comparisons between euploids and aneuploids for different morphological characters will be made later (see Aneuploidy in Section Four). Meanwhile it is of interest to know the extent to which the three populations differ from each other with respect to the relationship between seed-set and morphological characters. The heterogeneity tests for correlation coefficients in table II.7 give such information.

Except for plant height, there is no evidence of heterogeneity of correlation coefficients. The highly significant heterogeneity in the case of plant height suggests that the relationship of height with seed-set is variable. Therefore, it may be possible to select relatively shorter plants with high seed-set which, from the practical point of view, is important because shorter plants are less susceptible to lodging and this would help prevent consequent loss in grain yield.

B. Euploid Samples

I. The Sample Means for Morphological Characters

Forty euploid plants from each of the "high" and the unselected populations and twenty three euploids from the "low" populations were studied in detail for meiotic chromosome behaviour. These plants were also used for comparisons of morphological characters and seed-set. The sample means for each character for the three populations and their comparisons are presented in tables II.8a and II.8b respectively.

The difference in seed-set between euploid samples of "high" and "low" populations, unlike their random samples, is insignificant, although a reduced level of seed-set is observed in the "low" population. The unselected population has the highest seed-set but is not significantly different from the "high" population. If one takes into account the effect of heterosis in the unselected population, the seed-set in the high seed-set sample may well indicate some genetic gain as a result of selection. The heterosis is also observed in the unselected sample with regard to morphological characters, especially in plant height. On the other hand, the "low" population shows similar vigour as the unselected population with respect to number of tillers, number of spikelets and spike length, in spite of reduced seed-set. This suggests

Table II.8a. Means for Seed-set and Morphological Characters of Euploid Plants from High, Low and Unselected Populations, 1972

CHARACTERS	HIGH	LOW	UNSELECTED
	n = 40	n = 23	n = 40
Seed-set (Angular Values)	56.42±1.511	52.75±2.492	58.17±0.933
Plant height (cms)	127.58±2.553	133.91±3.235	141.75±1.615
No. of tillers per plant	5.73±0.318	7.52±0.790	7.50±0.479
No. of spikelets per spike	26.45±0.690	26.78±0.780	27.83±0.487
Spike length (cms)	10.72±0.256	11.36±0.248	11.85±0.206

Table II.8b. Comparisons of Euploid Plants from High, Low and Unselected Populations, 1972

CHARACTERS	POPULATIONS COMPARED	D.F. for separate variance estimate	t-value for separate variance estimate	Probability
Seed-set	(a) High/Low	38.24	1.26	n.s.
	(b) High/Unselected	64.96	0.98	n.s.
	(c) Low/Unselected	28.28	2.03	<0.05
Plant height	(a) High/Low	47.53	1.54	n.s.
	(b) High/Unselected	65.92	4.69	<0.001
	(c) Low/Unselected	33.18	2.17	<0.05
No. of tillers per plant	(a) High/Low	29.29	2.11	<0.05
	(b) High/Unselected	67.81	3.09	<0.01
	(c) Low/Unselected	38.24	0.02	n.s.
No. of spikelets per spike	(a) High/Low	51.93	0.32	n.s.
	(b) High/Unselected	70.14	1.63	n.s.
	(c) Low/Unselected	39.13	1.13	n.s.
Spike length	(a) High/Low	57.18	1.80	n.s.
	(b) High/Unselected	74.50	3.45	<0.001
	(c) Low/Unselected	49.37	1.52	n.s.

that increased plant vigour *per se* would not necessarily increase fertility. In other words, plant vigour without accompanying meiotic improvement would not be a reliable basis for increased seed-set. This is substantiated by the weak or lack of correlation of seed-set with morphological characters shown in table II.9.

Table II.9. Correlation Coefficients of Seed-Set with Morphological Characters in Euploid Samples of High, Low and Unselected Populations, 1972.

CHARACTERS	Correlation Coefficients			Heterogeneity Test for Correlation Coefficients		
	High	Low	Unselected	Chi-square	D.F.	P
Plant height	0.229	0.246	0.174	0.041	2	0.90-0.95
No. of tillers per plant	-0.066	0.085	0.005	-	-	-
No. of spikelets per spike	0.358*	0.153	0.005	2.691	2	0.30-0.50
Spike length	0.308*	-0.263	-0.058	4.867	2	0.05-0.10

* indicates significant at 5% level.

However, the heterogeneity of correlation coefficients of the three populations was insignificant which suggests that the populations do not differ in their relationships between seed-set and morphological characters.

II. Chromosome Associations in High, Low and Unselected Populations, 1972.

a. The Means

The means for different meiotic features of euploid plants in "high", "low" and unselected populations are presented in table II.10a and their comparisons in table II.10b. The sample means do not differ except for a significant increase in bivalent frequency ($P < 0.001$) with a corresponding decrease in quadrivalent frequency ($P < 0.001$) in the "high" population compared to the other two. The "high" population also differs from the unselected population by a significant increase in regular tetrads ($P < 0.05$). There is, however, no difference between the "low" and the unselected population in any of the meiotic features. Thus from the sample means, it is not clear that selection for meiosis regularity has been entirely successful.

From the theoretical point of view, chromosome doubling changes the nature of genetic segregation from disomic to tetrasomic inheritance. This greatly decreases the chance of getting a genotype homozygous at a particular locus. Irregular meiotic behaviour is apparently controlled by recessive factors with a complex basis of inheritance (Putt, 1954; Jones, 1967). Selection for recessive characters with a tetrasomic inheritance is an extremely slow process. Furthermore, a recessive

Table II.10a. Means for Different Meiotic Features and Seed-Set in High, Low and Unselected Populations, 1972.

CHARACTERS	HIGH	LOW	UNSELECTED
	n = 40	n = 23	n = 40
Chiasma Frequency per PMC	24.64±0.202	25.06±0.113	25.04±0.126
Cell-variance for chiasmata	2.78±0.182	2.30±0.141	2.36±0.141
Quadrivalent (IV) Frequency	2.20±0.063	2.72±0.073	2.68±0.040
Trivalent (III) Frequency	0.22±0.035	0.21±0.026	0.24±0.019
Bivalent (II) Frequency	9.05±0.123	8.05±0.166	8.09±0.088
Univalent (I) Frequency	0.44±0.055	0.36±0.04	0.39±0.039
No. of Chromosomes in IV + II Formations	26.90±0.154	27.00±0.116	26.92±0.092
No. of Chromosomes in III + I Formations	1.10±0.154	1.00±0.116	1.08±0.092
Disjunction Index (Angular Values)	59.64±1.781	61.50±2.001	58.39±1.350
Regular Tetrads (Angular Values)	68.07±0.803	66.96±0.724	65.48±0.788
Seed-set (Ang. Values)	56.42±1.511	52.75±2.492	58.13±0.931

Table II.10b. Comparisons of Meiotic Features in High, Low and Unselected Populations, 1972.

CHARACTERS	Population compared	D.F. for separate variance estimate	t-value for separate variance estimate	Probability
Chiasma Frequency	(a) High/Low	57.27	1.83	n.s.
	(b) High/Unselected	65.21	1.69	n.s.
	(c) Low/Unselected	59.07	0.14	n.s.
Cell-variance for chiasmata	(a) High/Low	60.94	2.08	<0.05
	(b) High/Unselected	73.39	1.84	n.s.
	(c) Low/Unselected	56.19	0.28	n.s.
Quadrivalent Frequency	(a) High/Low	51.06	5.44	<0.001
	(b) High/Unselected	66.30	6.46	<0.001
	(c) Low/Unselected	35.59	0.50	n.s.
Trivalent Frequency	(a) High/Low	61.00	0.06	n.s.
	(b) High/Unselected	59.43	0.52	n.s.
	(c) Low/Unselected	43.70	0.73	n.s.
Bivalent Frequency	(a) High/Low	45.21	4.85	<0.001
	(b) High/Unselected	70.76	6.38	<0.001
	(c) Low/Unselected	34.71	0.18	n.s.
Univalent Frequency	(a) High/Low	61.00	1.17	n.s.
	(b) High/Unselected	69.75	0.77	n.s.
	(c) Low/Unselected	53.55	0.52	n.s.
No. of chromosomes in IV + II formations	(a) High/Low	61.00	0.54	n.s.
	(b) High/Unselected	63.86	0.11	n.s.
	(c) Low/Unselected	47.95	0.57	n.s.
No. of chromosomes in III + I formations	(a) High/Low	61.00	0.54	n.s.
	(b) High/Unselected	63.84	0.11	n.s.
	(c) Low/Unselected	47.93	0.57	n.s.
Disjunction Index	(a) High/Low	52.18	0.69	n.s.
	(b) High/Unselected	72.70	0.56	n.s.
	(c) Low/Unselected	41.72	1.29	n.s.
Regular Tetrads	(a) High/Low	59.03	1.03	n.s.
	(b) High/Unselected	77.97	2.31	<0.05
	(c) Low/Unselected	58.58	1.38	n.s.

homozygote, being in this case semi-lethal or lethal, greatly limits the increase of recessive gene pool in the selected material. The individuals that normally survive are mostly heterozygotes and they will possess hybrid vigour. This may account for the insignificant differences between the low and the unselected populations.

The dominant traits in a tetrasomic inheritance are affected in a different way. Although their spread may actually be promoted by chromosome doubling (Stebbins, 1970), the main difficulty in demonstrating the effect of selection with dominant traits is the buffering tendency of the intermediate genotypes in the progenies. This also reduces the effect of genetic segregation. Therefore, any short term selection in a tetraploid population is least likely to show any measurable effects on sample means.

However, a more critical examination of the effect of selection, if any, can be made by comparing the regression coefficients of different populations. Such comparisons would show whether the functional relationships between any two variables described by the corresponding regression equations in different populations are the same. If they are not, this will suggest that the populations have diverged genetically, irrespective of any differences between the sample means. This is examined in the following chapter.

III. Inter-relationships of Meiotic Features

a. Chiasma Frequency and Other Meiotic Features

(i) Correlations Based on Plant Means

Quadrivalents: The relationship between quadrivalent and chiasma frequencies in "high", "low" and unselected populations are represented by the regression lines in figure II.1. In the high population the regression is positive and significant ($P < 0.05$) whereas in the low population it is negative but insignificant (Table II.11a). An intermediate relationship is observed with the unselected population. It, therefore, appears that first, the dependence of quadrivalents on chiasma frequency can be variable and, second, the relationship can even be negative. The latter, however, is surprising in view of the current concept that increasing chiasmata is associated with increased quadrivalent frequency (Roseweir & Rees, 1962; Hazarika & Rees, 1967) and, therefore, this warrants a careful consideration.

In autotetraploids quadrivalent formations ensure associations of the four homologous chromosomes by at least one chiasma between any two homologues. Failure of a single chiasma formation may leave one of the homologues unpaired as a univalent and the other three associated as a trivalent. It is on this basis that Hazarika & Rees (1967) and Roseweir &

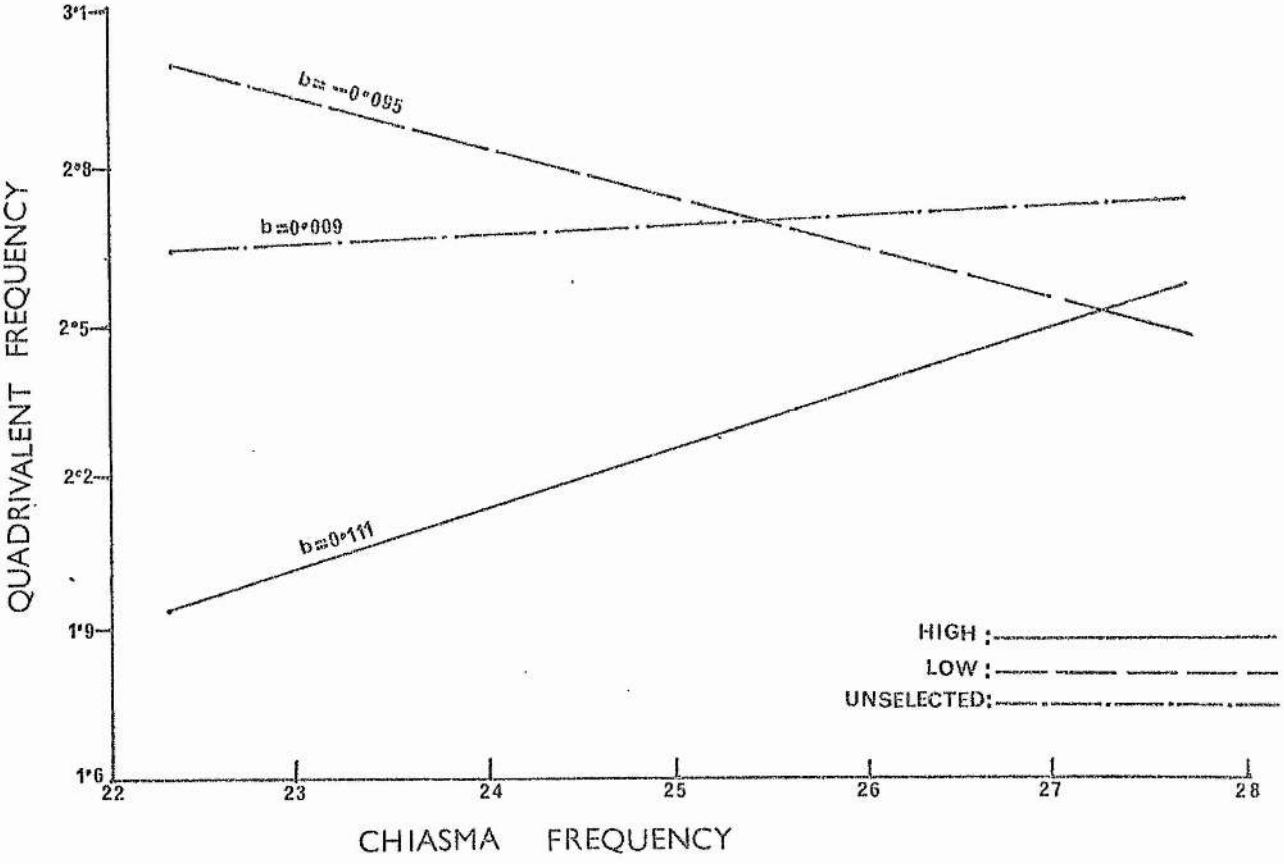


FIGURE II.1

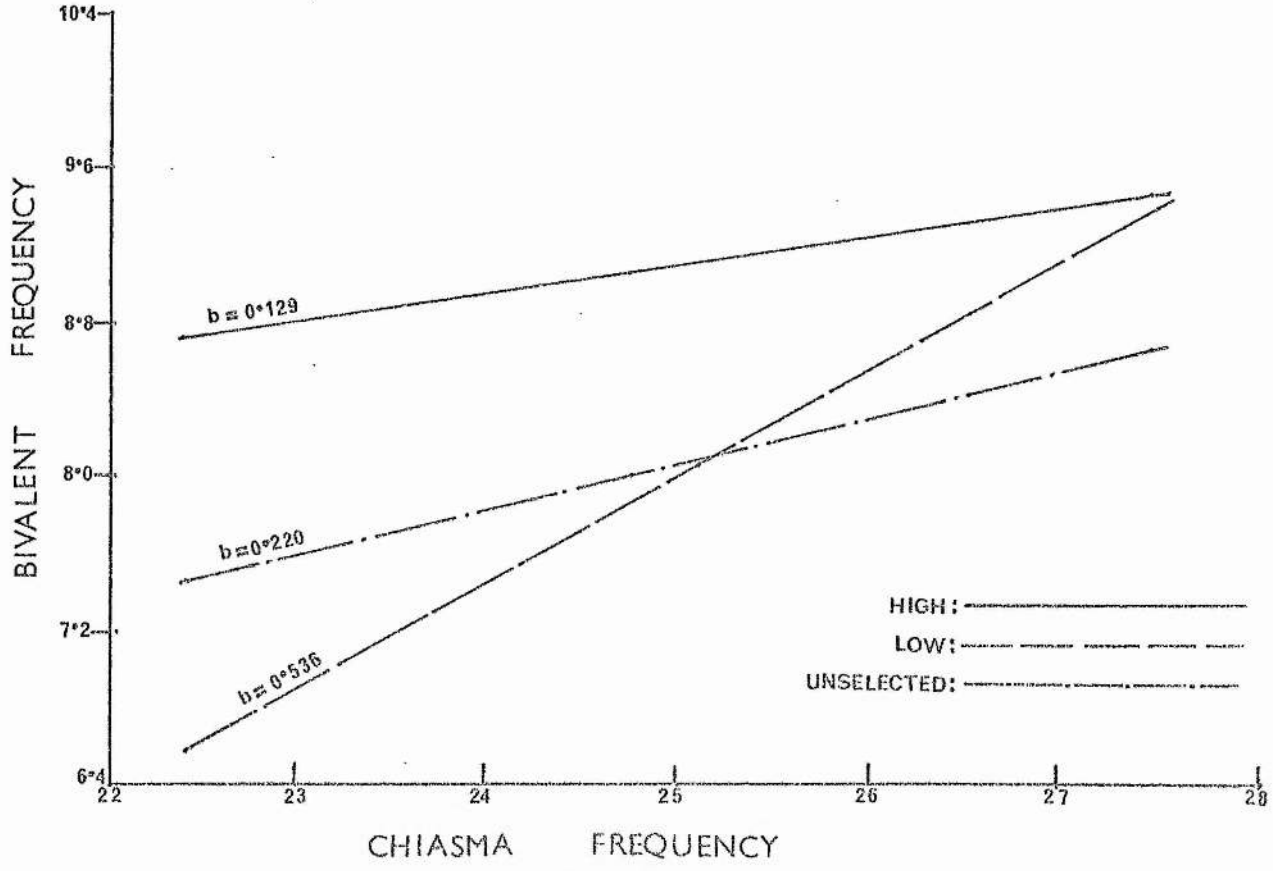


FIGURE II.2

Table II.11a. Variance Analyses of Regression of Quadrivalent Frequency on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F
Regression	1	0.787074	5.485*	1	0.05795	n.s. 0.459	1	0.00211	n.s. 0.032
Error	38	0.14350		21	0.12633		38	0.06699	

Table II.11b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.37494	0.18747	n.s. 1.724
Within Sample	97	10.54890	0.10874	
Total	99	10.92384		

* indicates significant at 5% level

n.s. " not significant

Rees (1962) demonstrated that chiasma frequency is positively correlated with quadrivalents and negatively with trivalents, bivalents and univalents. When such correlations are obtained one must assume that each of the seven sets of rye chromosomes shares, on an average, equal number of chiasmata. That is the distribution pattern of chiasmata throughout the genome is uniform.

This uniformity in the chiasma distribution pattern can be upset either by genic mutation (Jones, 1967 and 1974) or by structural changes in the chromosomes affecting chiasma formation (see Sybenga, 1969). When this happens, a higher frequency of unpaired chromosomes (i.e. univalents) will be observed at metaphase-I, the univalents subsequently form micronuclei in tetrads. In our "low" population, plants were selected for increased micronuclei in tetrads which means that at least two kinds of cytologically aberrant genotypes were favoured by the selection. These are:

(A) Genotypes with irregular chiasma distribution pattern in which the number of chiasmata in the seven sets of chromosomes is non-uniform (cf. "distributional mutant" described by Jones, 1964 and 1974).

(B) Genotypes with low chiasma frequency.

Now, as pointed out above, irregular chiasma distribution can be caused either by genic mutation (Jones, l.c.) or by

structural rearrangements in the chromosomes. The latter is synonymous to the "zygomere hypothesis" proposed by Sybenga (1966a). In the "low" population irregular chiasma distribution due to structural rearrangements seems to be a more probable cause because a higher frequency of plants with aberrant structures was detectable in this population, and, there were perhaps many more individuals in which the changes were cryptic and, therefore, cytologically undetectable. Evidently such individuals were favoured by irregular meiosis selection.

If in an autotetraploid, one of the four homologues had differentiated by structural rearrangements at one end of the chromosomes, chiasma formation at the re-arranged end will be inhibited and only the other end, being normal, will take part in chromatid exchange. In other words, when the rearranged chromosome is involved in pairing, chiasma frequency for this chromosome will be, on an average, half of its normal counterparts. By the same token, whenever the rearranged chromosome is involved in a quadrivalent formation, the number of chiasmata for that quadrivalent will be reduced. This will tend to give a negative correlation between quadrivalent frequency and chiasma frequency as observed in figure II.1. On the other hand, if the rearranged chromosome is left unassociated, the three normal counterparts will have a chance to form a maximum number of chiasmata in a trivalent.

[illegible] Possibly with favourable conditions for chiasma formation, trivalent association will be more frequent because of greater homology and therefore stronger affinity between the three normal counterparts and the rearranged chromosome will be left as a univalent. The same favourable conditions will encourage quadrivalent formations in other sets of chromosomes where the four homologues are identical. Therefore, one may also find a positive correlation between trivalent frequency and quadrivalent frequency in such genotypes (see figure II.13).

The similar relationships may also be observed in a genotype in which one of the four homologues is defective in exchange positions (pairing initiation points) in such a way that the potential pairing sites, instead of being localised at chromosome ends, are distributed throughout the chromosome length and the distributed points have uniform but low probability of satisfying exchange pre-conditions (see Jones, 1974).

Another reason for the negative correlation between chiasma frequency and quadrivalent frequency may be that the low population, being selected for irregular meiosis, have some plants with reduced number of chiasmata. If chiasma reduction is of the type of inbred lines, one may obtain higher quadrivalent frequency in spite of a reduction in chiasmata. This is not surprising because it was shown earlier that inbred lines have higher average of quadrivalents in spite of lower chiasma

frequency compared to outbred materials (see section I, table I.13).

It would, however, be unrealistic to suppose that the low population consists of only abnormal genotypes, as pointed out above. Because such genotypes normally would be poor in vigour and fertility, and natural selection will act against them. Although the frequency of abnormal genotypes might have increased as a result of selection, the bulk of the "low" population consists of normal genotypes. Consequently the negative correlation between chiasma frequency and the frequencies of quadrivalents would tend to be cancelled out so that there would be no apparent correlation in the population. This would be clear from the data in the appendix (Appendix Table IB) and also from the variance analysis of regression for the low population shown in table II.11 (see also appendix table 3 for correlation coefficients).

It should be borne in mind that in correlations based on plant means, the effects of individual genotypes are more acutely reflected than in a correlation computed disregarding individual plant means. That is, if the average configuration of all the plants in a population are plotted against the respective chiasma classes, the effects of abnormal genotypes, which are presumably very few, will be overshadowed by the predominant normal genotypes. In such a case, as will be shown later, the population chiasma classes will be positively

correlated with the average frequencies of quadrivalents.

Bivalents: Depending on the regression slope between quadrivalents and chiasmata, a related change in the slope between bivalents and chiasmata will be expected. This is because, firstly, bivalents are formed, in most cases, at the expense of quadrivalents and secondly, increases in chiasma frequency may occur either with increasing quadrivalents or with increasing bivalents, or with the increases in both. Therefore, as the regression slope between chiasmata and quadrivalents increases, the slope between bivalents and chiasmata decreases until the latter becomes negative as shown in inbred materials (Hazarika, 1966). This becomes clear when figures II.1 and II.2 are compared. For example, of the three populations, the "high" group shows the highest regression coefficient between quadrivalent frequency and chiasma frequency, in contrast to its lowest regression coefficient between bivalents and chiasmata. Similar related variations can also be observed with respect to the "low" and the unselected populations.

From figure II.2 it will be seen that bivalent frequency is positively related with the frequencies of chiasmata in each of the three populations. The variance analyses in table II.12a shows that the regression is insignificant in the high population but significant in both the low and the unselected populations ($P < 0.05$).

Table II.12a. Variance Analyses of Regression of Bivalent Frequency on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1.05433	n.s. 1.777	1	1.85680	* 3.244	1	1.19065	* 4.124
Error	38.	0.59326		21	0.57236		38	0.28869	

Table II.12.b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	1.03408	0.51704	n.s. 1.101
Within Sample	97	45.54922	0.46958	
Total	99	46.58330		

* indicates significant at 5% level

n.s. " not significant

However, both with quadrivalents and bivalents, the heterogeneity between regression slopes is not significant (Tables II.11b and II.12b). This means that the populations cannot yet be regarded as distinctly different from each other with respect to their pairing pattern. In view of the short period of selection and the complex genetic basis of pairing pattern (Rees & Thompson, 1956; Jones, 1967 and 1974) and also the rather slow approach to homozygosity with tetrasomic inheritance the diverging tendency exhibited by the populations is nonetheless important.

Trivalents and Univalents: In eutetraploids, for every trivalent formed, there is one chromosome left unpaired as a univalent. Therefore, there is a strong positive correlation between trivalent frequency and univalent frequency (appendix Table 3) and both these two configurations are negatively correlated with chiasma frequency (figures II.3 and II.4). The separate analyses of variance in table II.13a and II.14a show that the regressions of trivalent and of univalent frequencies on chiasmata are significant in each of the three populations.

In the case of trivalents, the heterogeneity between regression slopes of the populations is significant ($P < 0.05$, table II.13b). This suggests that the rates of decrease in trivalents with increasing chiasmata vary between populations.

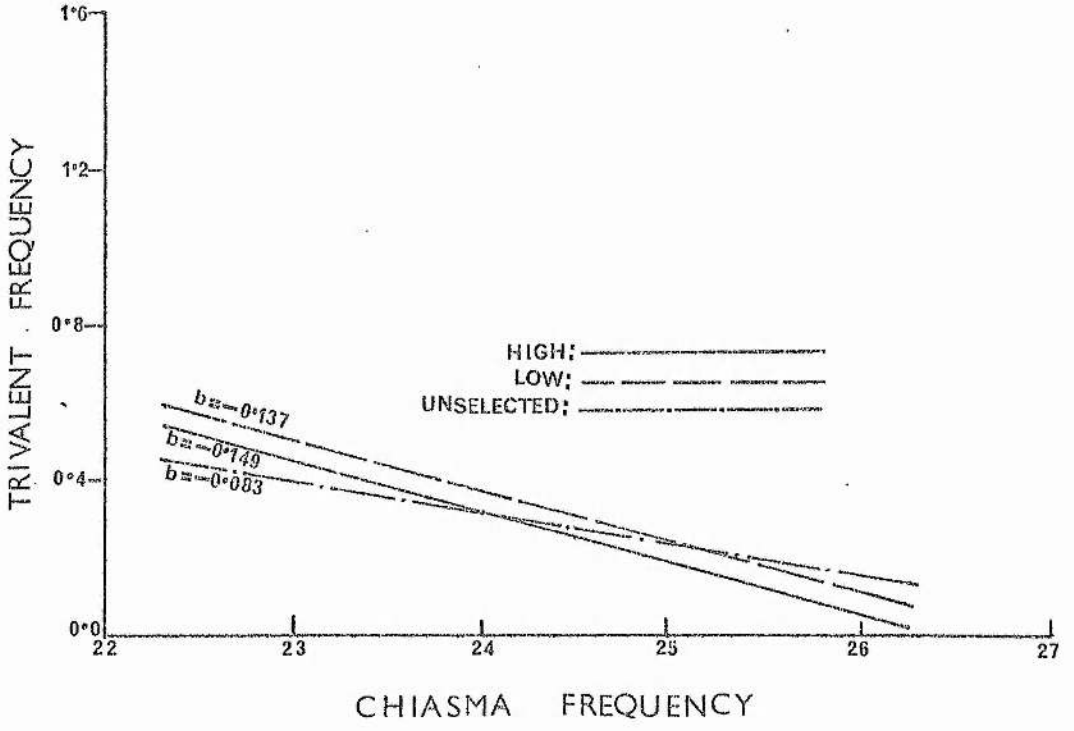


FIGURE II.3

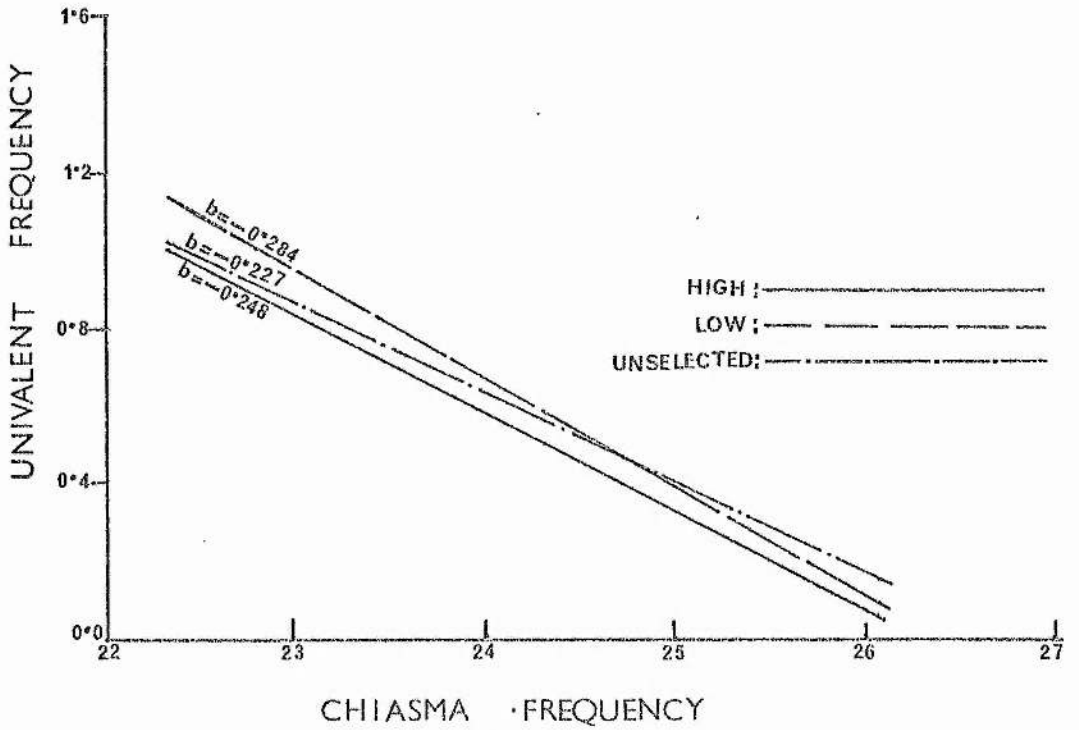


FIGURE II·4

Table II.13a. Variance Analyses of Regression of Trivalent Frequency on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1.42290	115.565***	1	0.12048	11.468***	1	0.17060	17.794***
Error	38	0.01231		21	0.01051		38	0.00959	

Table II.13b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.08189	0.04095	3.764*
Within Sample	97	1.05502	0.01088	
Total	99	1.13691		

*** indicates significant at 0.1% level

* " " at 5% level

Table II.14a. Variance Analyses of Regression of Univalent Frequency on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	3.90413	165.034***	1	0.51995	29.370***	1	1.27033	44.942***
Error	38	0.02366		21	0.01770		38	0.02827	

Table II.14b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.03154	0.01577	0.590 n.s.
Within Sample	97	2.59159	0.02672	
Total	99	2.62313		

*** indicates significant at 0.1% level

n.s. " not significant

This is not surprising in view of the presence of aberrant genotypes, postulated earlier, in the low population.

In the case of univalents the heterogeneity of regression slopes, unlike that of trivalent frequency, is insignificant. These results are not in fact incompatible because there are two sources of univalent formations. One is associated with the failure of quadrivalent formation giving rise to one trivalent and one univalent and the other source is the failure of a bivalent formation giving rise to two univalents. It is because of the latter that the correlation coefficient between the frequencies of trivalents and univalents is always less than unity (appendix table 3). However, the insignificant heterogeneity between regression slopes suggest that the overall univalent frequency, arising from both sources, has the same dependence on chiasma frequency in the three populations.

Number of Chromosomes in (IVs + IIs) and (IIIs + Is).

It has been shown above that the increases in chiasma frequency can lead to the increases in IVs and/or IIs, but depending on the regression slope between chiasmata and quadrivalents, there is a related change in the slope between chiasmata and bivalents. This creates a confusion about the effect of chiasma frequency on pairing regularity. This can be avoided by combining the frequencies of IVs and IIs and

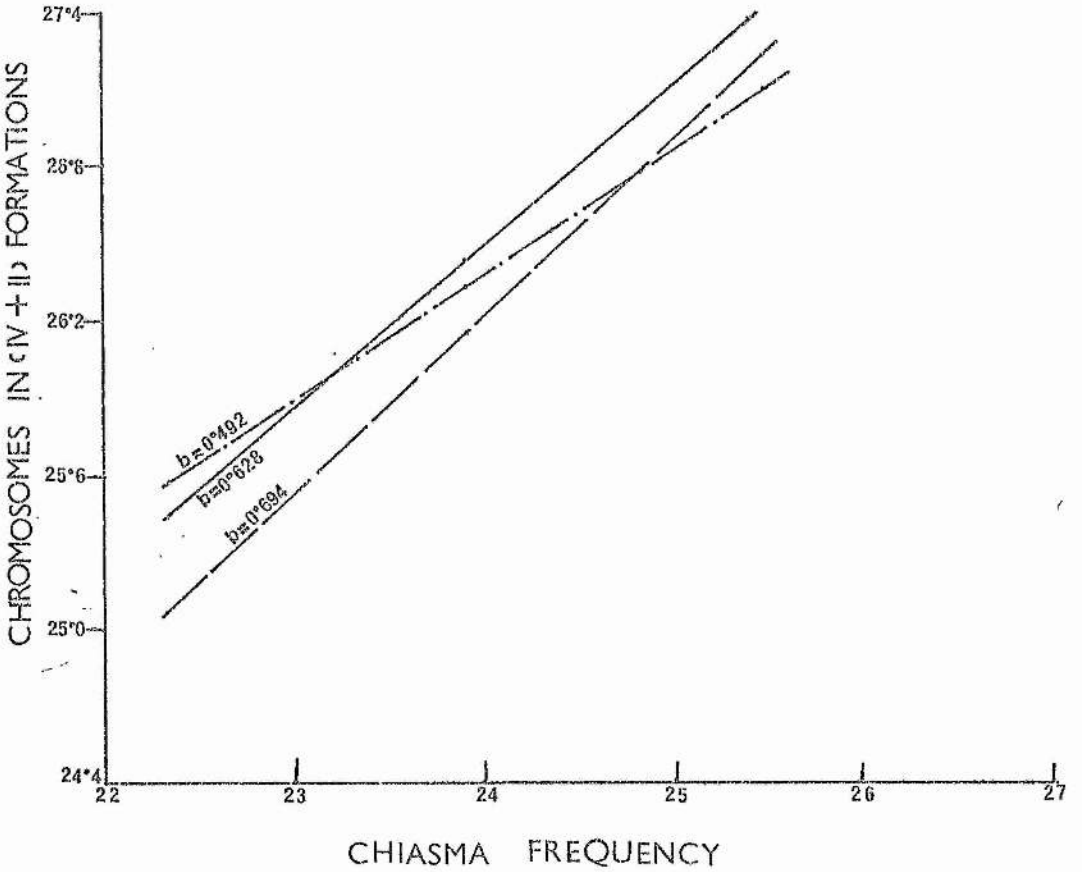


Figure II.5

High: ——————————
Low: — — — — — —
Unselected: ——·——·——

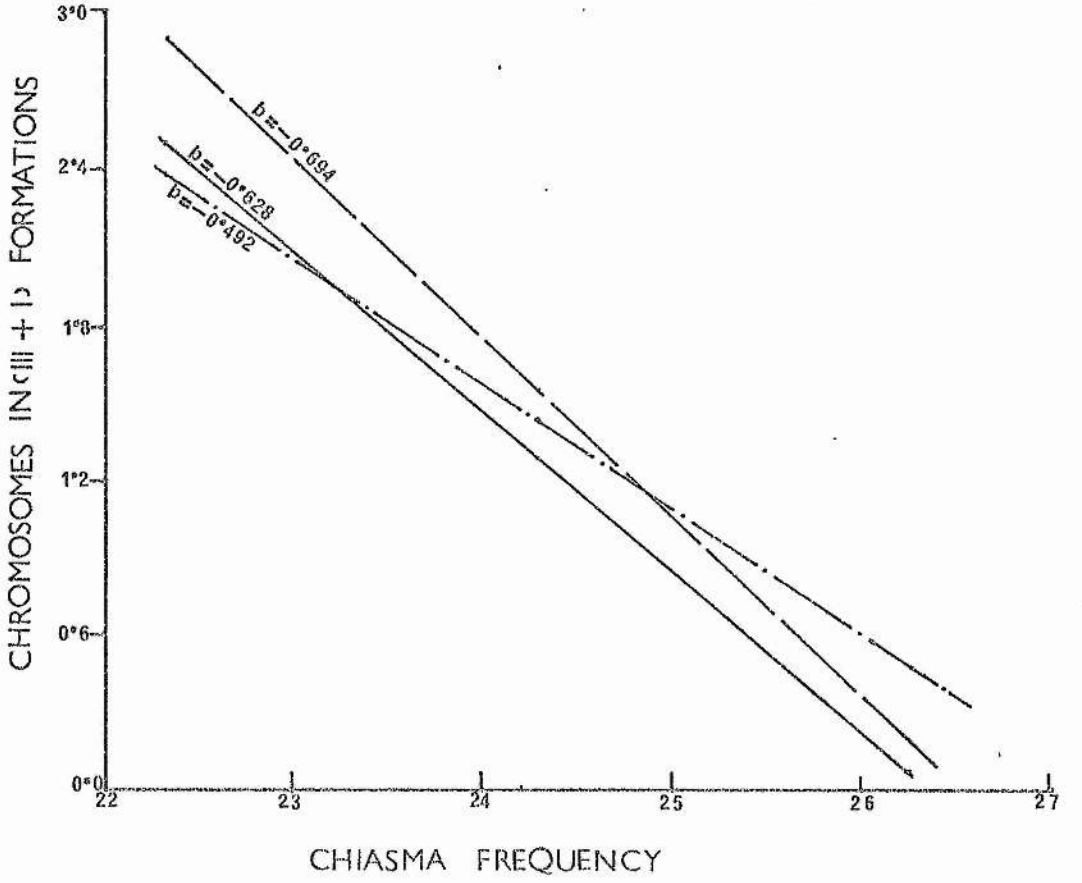


Figure II.6

plotting the total number of chromosomes involved in these two configurations against chiasma frequency. This is shown in figure II.5 for the three populations. In each population the regression is significant (table II.15a).

In a plant the number of chromosomes involved in (IVs + IIs) is measured against the number of chromosomes involved in (IIIs + Is). Therefore, the increase in chromosome numbers in (IVs + IIs) is at the same rate as the decrease in chromosome numbers in (IIIs + Is). One would consequently expect chiasma frequency to be correlated at the same magnitude with chromosomes involved in (IVs + IIs) and (IIIs + Is), except that the correlation with the former is positive and with the latter it is negative. This is evident from figures II.5 and II.6 where the magnitude of the two regression coefficients for a particular population is the same (see also their correlation coefficients in appendix table 3).

The advantages of combining metaphase-I configurations as above are manifold. Firstly, as already mentioned, this avoids the anomaly caused by related changes in the frequencies of quadrivalents and bivalents. Secondly, one correlation coefficient, instead of four, is needed to get an index of pairing regularity, the index being comparable to "disjunction index" suggested by Hazarika & Rees (1967). Thirdly, the correlation is computed from actual figures rather than

Table II.15a. Variance Analyses of Number of Chromosomes Involved in (IVs + IIs) on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	25.11290	81.004***	1	3.10599	17.790***	1	5.95601	30.940***
Error	38	0.31002		21	0.17459		38	0.19250	

Table II.15b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.47258	0.23629	1.007 n.s.
Within Sample	97	22.76207	0.23466	
Total	99	23.23465		

** indicates significant at 1% level

*** " " at 0.1% "

n.s. " not significant

Table II.16a. Variance Analyses of Regressions of Number of Chromosomes Involved in (IIIs + Is) on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	25.11282	81.004***	1	3.10599	17.790***	1	5.95601	30.940***
Error	38	0.31002		21	0.17459		38	0.19250	

Table II.16b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.47258	0.23629	1.007 n.s.
Within Sample	97	22.76219	0.23466	
Total	99	23.23477		

*** indicates significant at 0.1% level

n.s. " not significant

percentages and their transformed values as is necessary with "disjunction index" and it thus ensures greater accuracy than the "disjunction index".

The heterogeneity test for the regression slopes in the three populations was not significant for the number of chromosomes involved in (IVs + IIs) or in (IIIs + Is) (tables II.15b and II.16b). This again suggests that the populations do not differ in their pairing pattern.

Disjunction Index and Regular Tetrads

Both disjunction index and regular tetrads, as will be shown later, are directly dependent on the frequencies of quadrivalents and bivalents. The latter two, in turn, correlate positively with chiasma frequency. We would, therefore, expect a positive correlation between chiasma frequency on one hand and disjunction index and regular tetrads on the other. Figures II.7 and II.8 demonstrate this for the three populations. The regression is significant in each population (tables II.17a and II.18a) but not the heterogeneity between regression slopes (tables II.17b and II.18b). From this one concludes that with the increases in chiasma frequency there are increases in both disjunction index as well as regular tetrads and the corresponding rate of change is the same for the three populations.

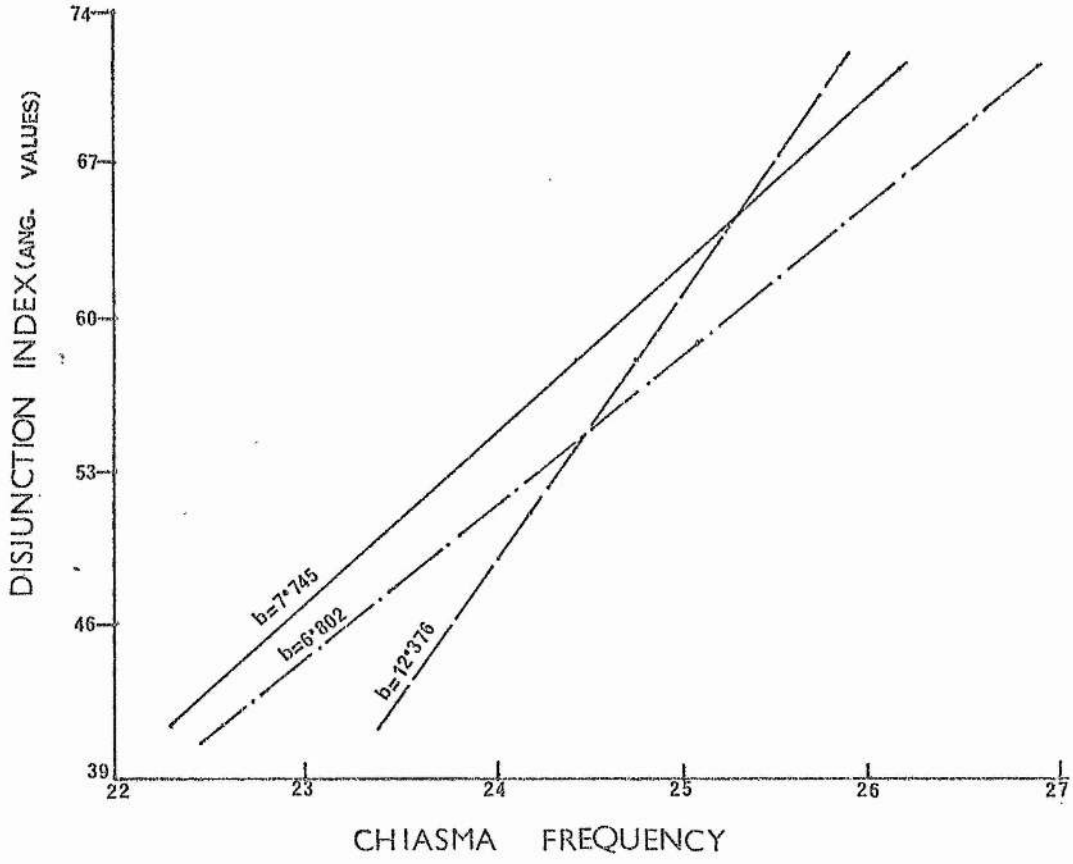


FIGURE II.7

HIGH:
LOW:
UNSELECTED:

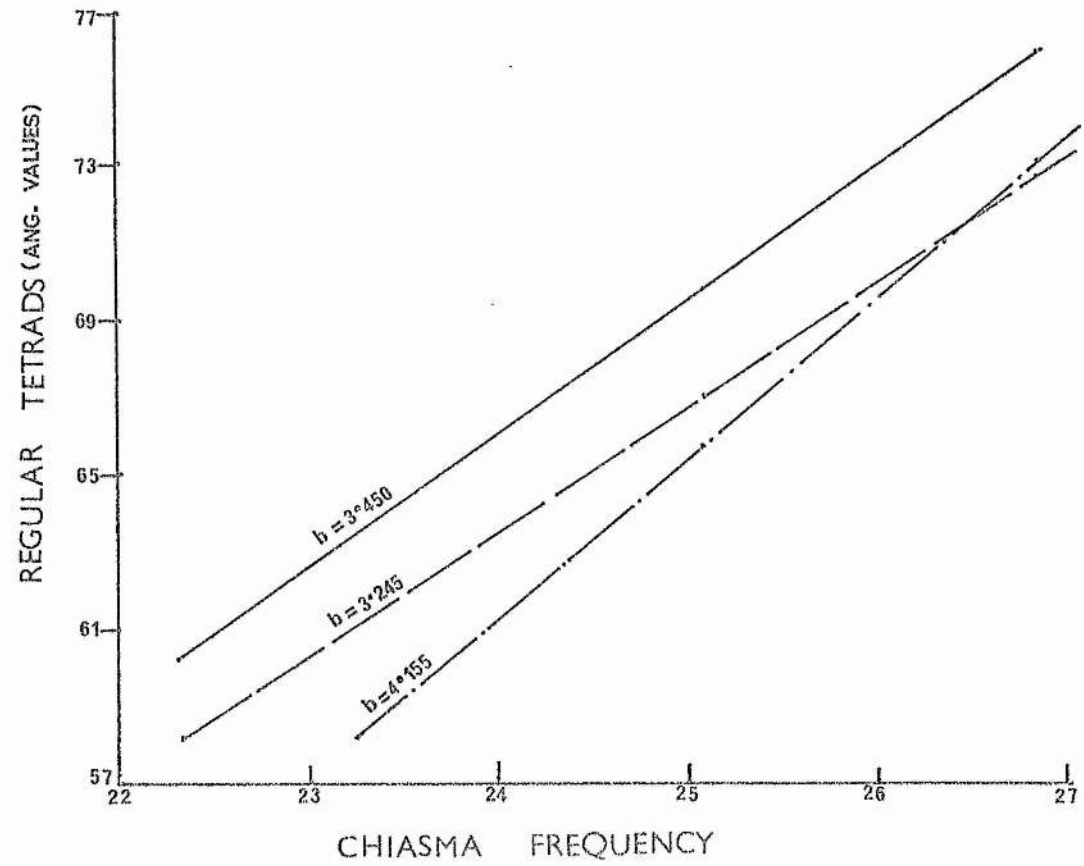


FIGURE II.8

Table II.17a. Variance Analyses of Regression of Disjunction Index on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	3818.13971	128.263***	1	988.42986	19.990***	1	1136.97782	25.294***
Error	38	29.76810		21	49.44665		38	44.95038	

Table II.17b. Variance Analysis of Heterogeneity of Regression between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	173.72704	86.86352	2.173 n.s.
Within Sample	97	3877.68261	39.97611	
Total	99	4051.40965		

*** indicates significant at 0.1% level

n.s. " not significant

Table II.18a. Variance Analyses of Regular Tetrads on Chiasma Frequency in High, Low and Unselected Populations, 1972

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	757.75585	115.678***	1	67.94610	7.225**	1	423.30115	29.675***
Error	38	6.55058		21	9.40423		38	14.29812	

Table II.18b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	12.87383	6.43692	0.638 n.s.
Within Sample	97	978.10790	10.08359	
Total	99	990.98173		

** indicates significant at 1% level

*** " " at 0.1% level

n.s. " not significant

In general, the correlations based on plant means confirm the findings of earlier workers that in tetraploid rye high chiasma frequency increases the frequencies of those configurations that ensure regular chromosome separation. In inbred lines this has been shown to be achieved by an increase in quadrivalent frequency and a decrease in the frequencies of trivalents, bivalents and univalents (Roseweir & Rees, 1962; Hazarika & Rees, 1967). The three populations investigated here seemed to possess variable relationship between chiasma frequency on one hand and the frequencies of quadrivalents and bivalents on the other. The "low" and the unselected populations showed significant positive correlations with bivalent frequency while in the "high" population, the correlation was significant with quadrivalent frequency. This indicates that the increases in chiasma frequency can influence the pairing pattern in two different ways, i.e. either by increasing the frequency of quadrivalents or by increasing the frequency of bivalents. When the chiasma distribution pattern is predominantly "free" within the four homologous chromosomes, there is a greater tendency to form quadrivalents with increasing chiasmata. On the other hand, with "restricted" pairing pattern, the four representative chromosomes may pair in a two-by-two manner forming bivalents. Such variations in chiasma distribution pattern was indeed realised by Hazarika & Rees (l.c.). They observed that for comparable chiasma frequency, the genotypes differed significantly in their average pairing configurations. This

led them to conclude that the distribution pattern of chiasmata, like chiasma frequency, is a heritable component.

A similar comparison between the present populations can be made by considering the chiasma classes within a population on one hand and, on the other, average pairing configurations under each chiasma class, irrespective of individual plant means. Such analyses would reveal if the populations differ with respect to pairing behaviour that are independent of chiasma frequency. This is shown by correlations based on cell population means.

(ii) Correlations Based on Cell Population Means

In the following analyses, therefore, all the pollen mother cells with the same chiasma frequency have been grouped together from all the plants within a population. The average frequencies of metaphase-I configurations under respective chiasma class in "high", "low" and unselected populations are presented in table II.19.

Quadrivalents

Figure II.9 shows the relationship between chiasma classes and average quadrivalent frequency in the three populations. The regression is positive in each population.

Table II.19. Mean Frequencies of Different Metaphase-I Configurations under Respective Chiasma Classes within High, Low and Unselected Populations, 1972.

Chiasma Classes in PMC	IVs			IIIs			IIs			Is		
	HIGH	LOW	UNSELECTED	HIGH	LOW	UNSELECTED	HIGH	LOW	UNSELECTED	HIGH	LOW	UNSELECTED
16	2.00	-	-	2.33	-	-	6.00	-	-	3.67	-	-
18	1.20	-	-	1.60	-	-	6.60	-	-	3.60	-	-
19	1.00	-	3.00	0.80	-	1.00	9.60	-	6.00	2.40	-	1.00
20	1.55	1.00	2.00	0.73	0.00	1.14	9.73	12.00	7.43	2.00	0.00	1.71
21	1.78	2.50	2.41	0.39	2.00	0.47	9.22	8.50	7.76	1.26	1.50	1.41
22	1.77	2.61	2.59	0.51	4.00	0.41	9.64	7.09	7.83	1.23	1.26	1.05
23	2.23	2.66	2.61	0.22	0.65	0.41	8.81	7.88	7.77	0.61	0.48	0.80
24	2.30	2.61	2.57	0.23	0.42	0.32	8.97	8.18	8.22	0.46	0.51	0.52
25	2.07	2.81	2.69	0.16	0.30	0.26	9.30	7.79	8.27	0.28	0.35	0.35
26	2.28	2.72	2.70	0.14	0.13	0.14	9.18	8.25	8.12	0.16	0.22	0.16
27	2.50	2.90	2.89	0.02	0.05	0.07	8.81	8.35	7.96	0.04	0.07	0.07
28	2.58	2.60	2.78	0.03	0.00	0.02	8.52	8.10	8.10	0.03	0.00	0.02

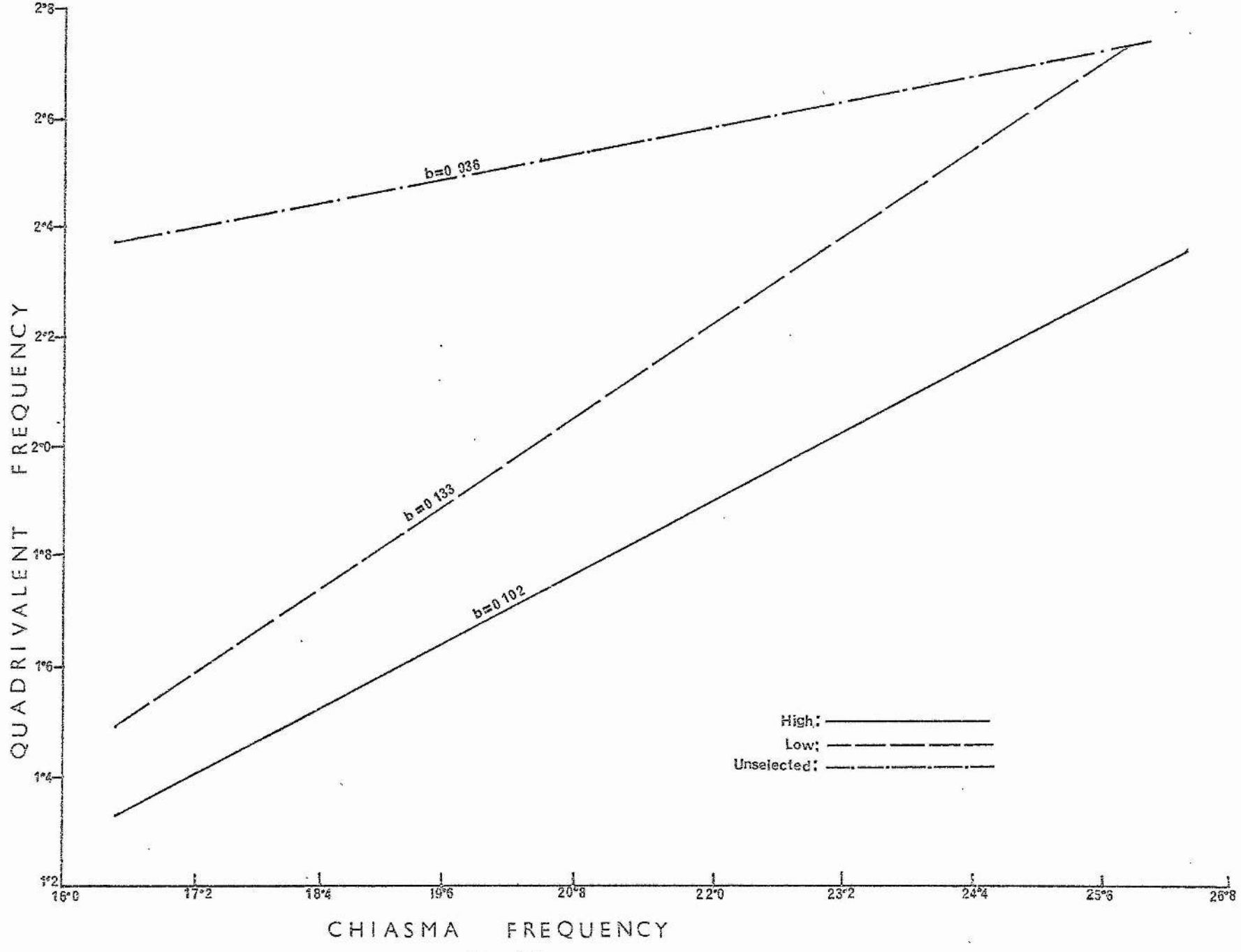

2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
QUADRIVALENT FREQUENCY
b=0 036
b=0 133
b=0 102
High:
Low:
Unselected:
16·0
17·2
18·4
19·6
20·8
22·0
23·2
24·4
25·6
26·8
CHIASMA FREQUENCY

Figure II·9

The variance analyses in table II.20a show that in the "high" and the "low" populations the regressions are significant at 1% and 5% level respectively whereas in the unselected population the rgression is insignificant. This again points to the variable relationship observed earlier with plant means. It will, however, be noted that in the low population the correlation based on plant means was negative (figure II.1) whereas the correlation here, based on population means, is positive. This supports the view that the low population predominantly consists of normal genotypes.

The heterogeneity in regression slopes is, however, insignificant (table II.20b). This suggests that the populations do not in fact behave differently with respect to the dependence of quadrivalents on chiasma frequency. But the heterogeneity of means is significant ($P < 0.01$) (table II.20b) indicating that for similar chiasma frequency there are different quadrivalent frequencies in the three populations. Clearly this difference in the frequencies of quadrivalents between populations is independent of chiasma frequency but is due to the variation in chiasma distribution pattern as suggested by Hazarika & Rees (1967).

Bivalents

It will be seen in figure II.10 that bivalent frequency is positively correlated with chiasma classes in the high and

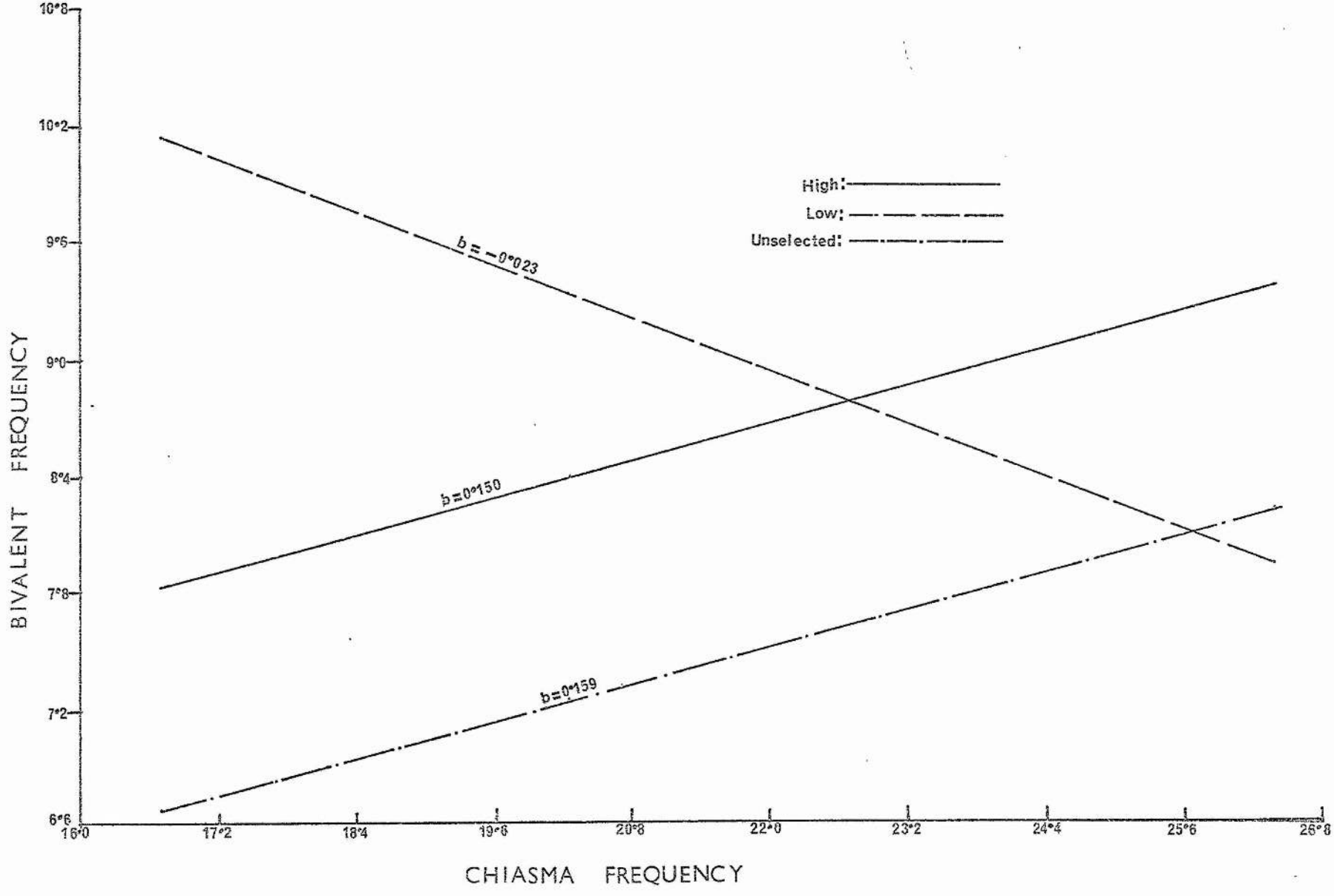

10·8
10·2
9·6
9·0
8·4
7·8
7·2
6·6
BIVALENT FREQUENCY
16·0
17·2
18·4
19·6
20·8
22·0
23·2
24·4
25·6
26·8
CHIASMA FREQUENCY
High:
Low:
Unselected:
b = −0·023
b=0·150
b=0·159

Figure II.10

Table II.20a. Variance Analyses of Regression of Quadrivalent Frequency on Chiasma Classes in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1.60499	14.515**	1	1.05868	4.767*	1	0.10764	1.489 n.s.
Error	10	0.11058		7	0.22210		8	0.07230	

Table II.20b. Variance Analysis of Heterogeneity of Regressions and Means between Populations.

ITEMS	D.F	S.S.	M.S.	F
Between Regressions	2	0.372	0.189	1.454 n.s.
Between Means	2	1.952	0.976	7.508**
Error	25	3.238	0.130	
Total	29	5.562		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

Table II.21a. Variance Analyses of Regression of Bivalent Frequency of Chiasma Classes in High, Low and Unselected Populations, 1972

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	3.46490	2.892	1	3.18321	1.815 n.s.	1	2.07695	8.794 *
Error	10	1.19799		7	1.75377		8	0.23619	

Table II.21b. Variance Analysis of Heterogeneity of Regressions and Means between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	7.032	3.516	3.361 +
Between Means	2	5.821	2.911	2.783 n.s.
Error	25	26.146	1.046	
Total	29	38.999		

* indicates significant at 5% level

** " " at 1% level

*** " " at 0.1% level

n.s. " not significant

\+ " almost significant at 5% level

the unselected populations, the regression is significant only in the case of the unselected population ($P < 0.01$, see table II.21a). In contrast there is a negative relationship in the low population, the rgression, however is insignificant, (table II.21a). The heterogeneity between regression slopes is almost significant ($P = 0.05$, table II.21b). It thus appears that with increasing chiasma frequencies, the frequencies of bivalents tend to vary in different populations. The increase is the highest in the unselected population, insignificant in the high and there is a decrease in the low population. This can only be explained in terms of differences in chiasma distribution pattern between populations.

The unselected population, being highly heterozygous, favours more restricted pairing to form bivalents as compared to the "low" and the "high" populations. Again it will be noted that in the low population the regression between chiasma frequency and bivalents, when based on plant means, was positive (figure II.2) but the regression, based on cell population means in figure II.10, is negative. This is because, as pointed out earlier, the increasing number of bivalents in the abnormal genotypes, where one of the four homologues has differentiated at one end, means a greater participation of the rearranged chromosome in bivalent formations. These bivalents, being rods, reduce the average number of chiasmata in the PMC. This has been realised

in the correlation based on population mean because when all bivalents are grouped together, the higher average of bivalents per PMC includes a higher number of rod bivalents formed by the rearranged chromosome(s). Whereas in correlation based on individual plant means such effects remain hidden due to the predominant normal genotypes which most frequently form ring bivalents.

Trivalents

The relation between trivalent frequency and chiasma classes in the three populations has been shown in figure II.11. As expected, the regression is negative in each population. The variance analyses in table II.22a show that the regressions in the high and the unselected populations are highly significant ($P < 0.01$) but insignificant in the case of the "low" population. There is, however, no significant heterogeneity between regression coefficients or between population means. Therefore, with respect to trivalent frequency and its dependence on chiasma frequency, the populations are statistically similar. On the other hand, the insignificant regression in the "low" population tends to support that the dependence of pairing configurations on chiasma frequency can be variable.

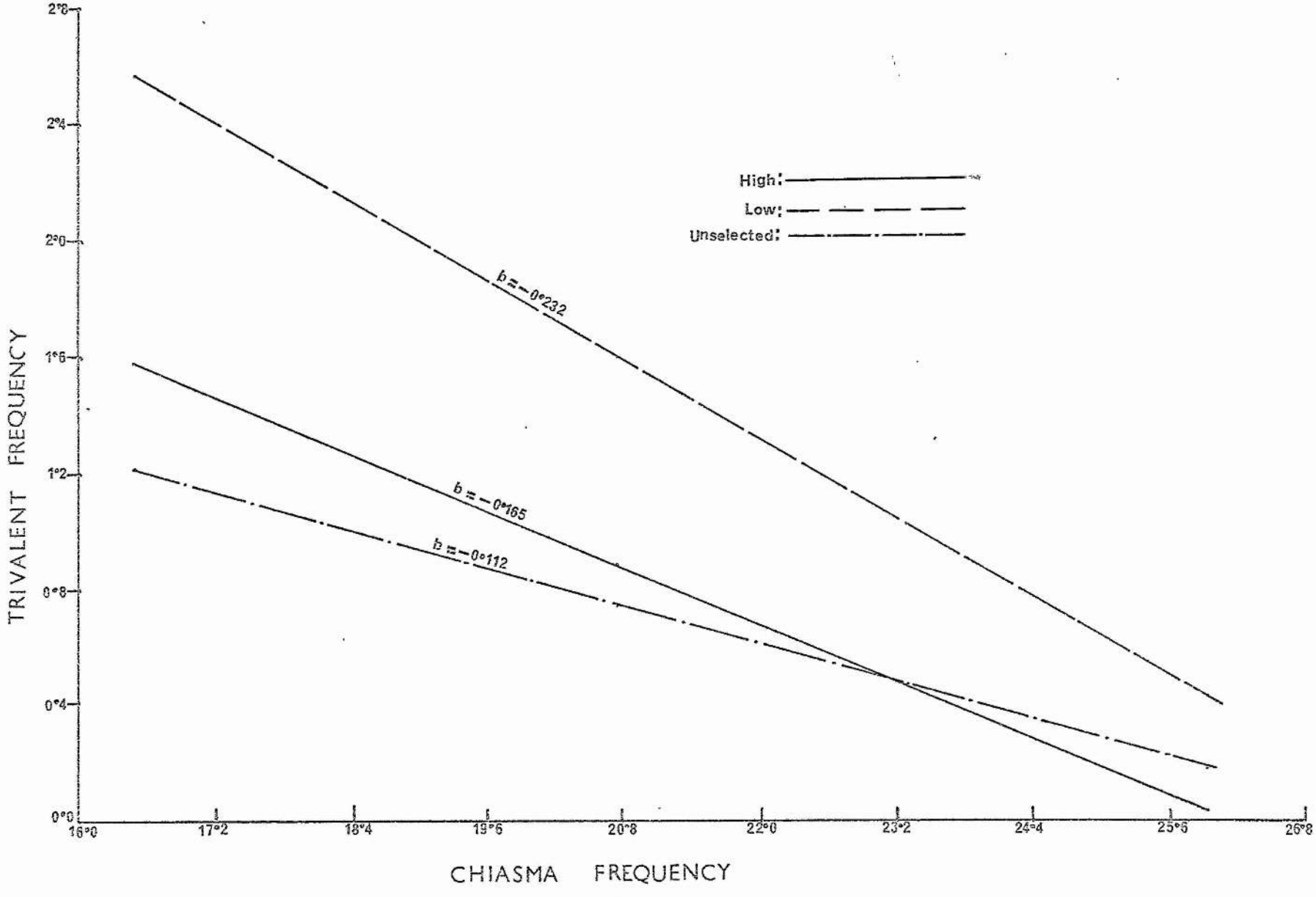

High:
Low:
Unselected:
b = −0·232
b = −0·165
b = −0·112
TRIVALENT FREQUENCY
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0·0
16·0
17·2
18·4
19·6
20·8
22·0
23·2
24·4
25·6
26·8
CHIASMA FREQUENCY

Figure II·11

Table II.22a. Variance Analyses of Regression of Trivalent Frequency on Chiasma Classes in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F.	D.F.	M.S.	F
Regression	1	4.23481	34.859***	1	3.23873	2.036 n.s.	1	1.03712	38.285***
Error	10	0.12149		7	1.59085		8	0.02709	

Table II.22b. Mariance Analysis of Heterogeneity of Regressions and Means between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.503	0.252	$0.509^{n.s.}$
Between Means	2	1.752	0.875	$1.768^{n.s.}$
Error	25	12.373	0.495	
Total	29	14.628		

*** indicates significant at 0.1% level

n.s. " not significant

Univalents

The regressions of univalent frequency on chiasma classes in the three populations are shown in figure II.12 and their variance analyses in table II.23a. Like trivalents, the univalents have a negative relationship with chiasma classes in each population. The regression is highly significant for the high and the unselected populations ($P < 0.001$) and insignificant in the low population. The heterogeneity between regression slopes is highly significant ($P < 0.01$, table II.23b) which confirms that the dependence of pairing configurations on chiasma frequency is different for the populations.

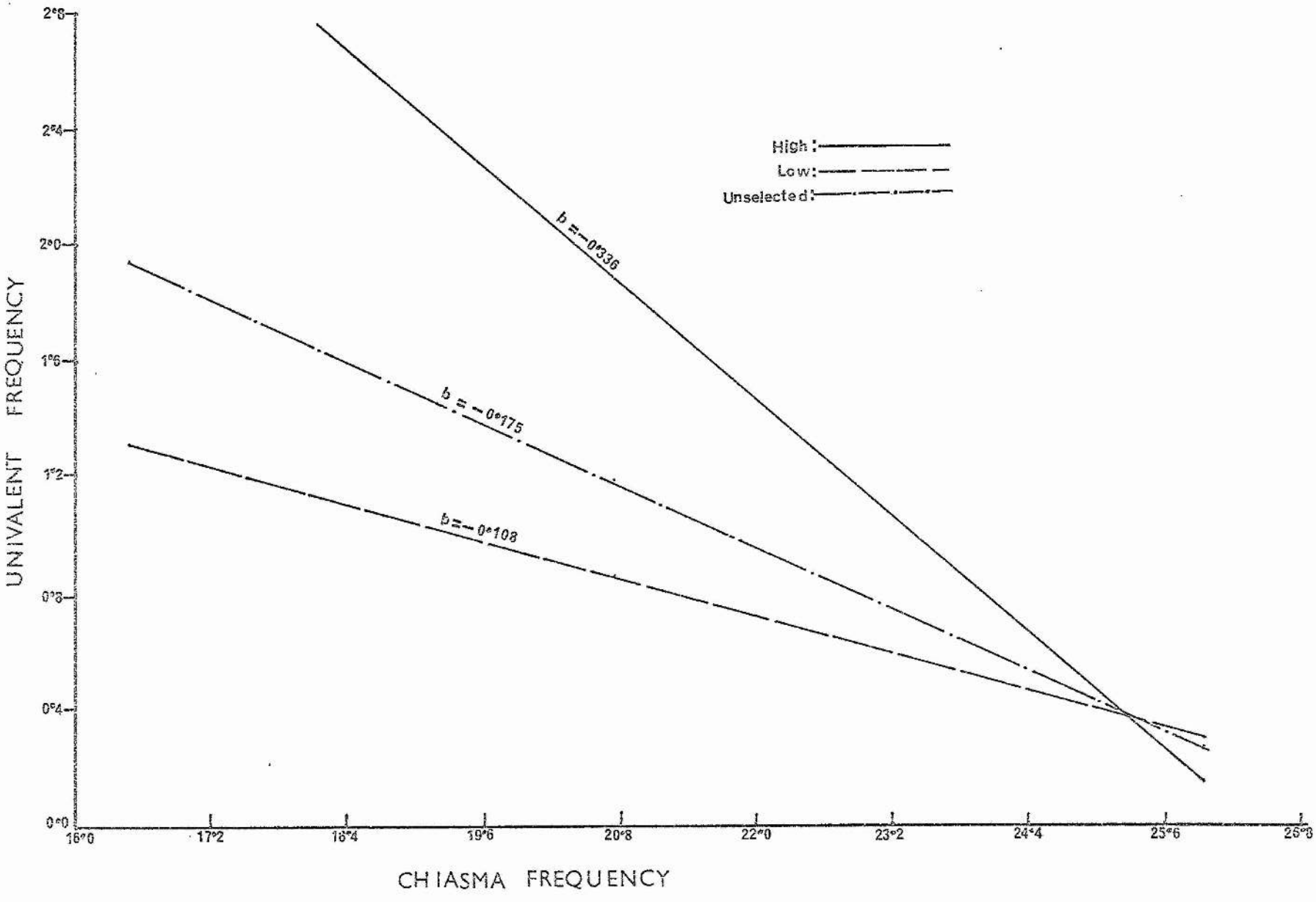

High:
Low:
Unselected:
b = −0·336
b = −0·175
b = −0·108
UNIVALENT FREQUENCY
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0·0
16·0
17·2
18·4
19·6
20·8
22·0
23·2
24·4
25·6
26·8
CHIASMA FREQUENCY

Figure II.12

Table II.23a. Variance Analyses of Regression of Univalent Frequency on Chiasma Classes in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	17.48700	94.680***	1	0.70417	2.972 n.s.	1	2.53620	39.360***
Error	10	0.18470		7	0.23691		8	0.06444	

Table II.23b. Variance Analysis of Heterogeneity of Regressions and Means between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	2.832	1.416	8.795**
Between Means	2	1.017	0.509	3.161 n.s.
Error	25	4.024	0.161	
Total	29	7.973		

** indicates significant at 1% level

*** " " at 0.1% level

n.s. " not significant

b. Quadrivalent Frequency and Other Meiotic Features

The three populations considered in the investigation seem to differ in chiasma distribution pattern. As a result, one would also expect differences in the inter-relationships of metaphase-I configurations and the subsequent meiotic features. The following analyses were, therefore, made to examine such changes in different populations. The analyses were based on plant means.

Trivalents

Let us first consider the relationship between trivalent frequency and quadrivalent frequency. Figure II.13 shows the regression between the two configurations in "high", "low" and unselected populations and table II.24a gives their regression analyses. As expected, the regression in the high population is negative and significant ($P < 0.05$) whereas in the low population the regression is positive, although insignificant.

In the unselected population, the regression line is almost horizontal which indicates that there is virtually no change in quadrivalent frequency as a result of changes in trivalent frequency. This was explained earlier as due partly to the "equilibrium state" of the population with regard to its pairing behaviour and partly to restricted

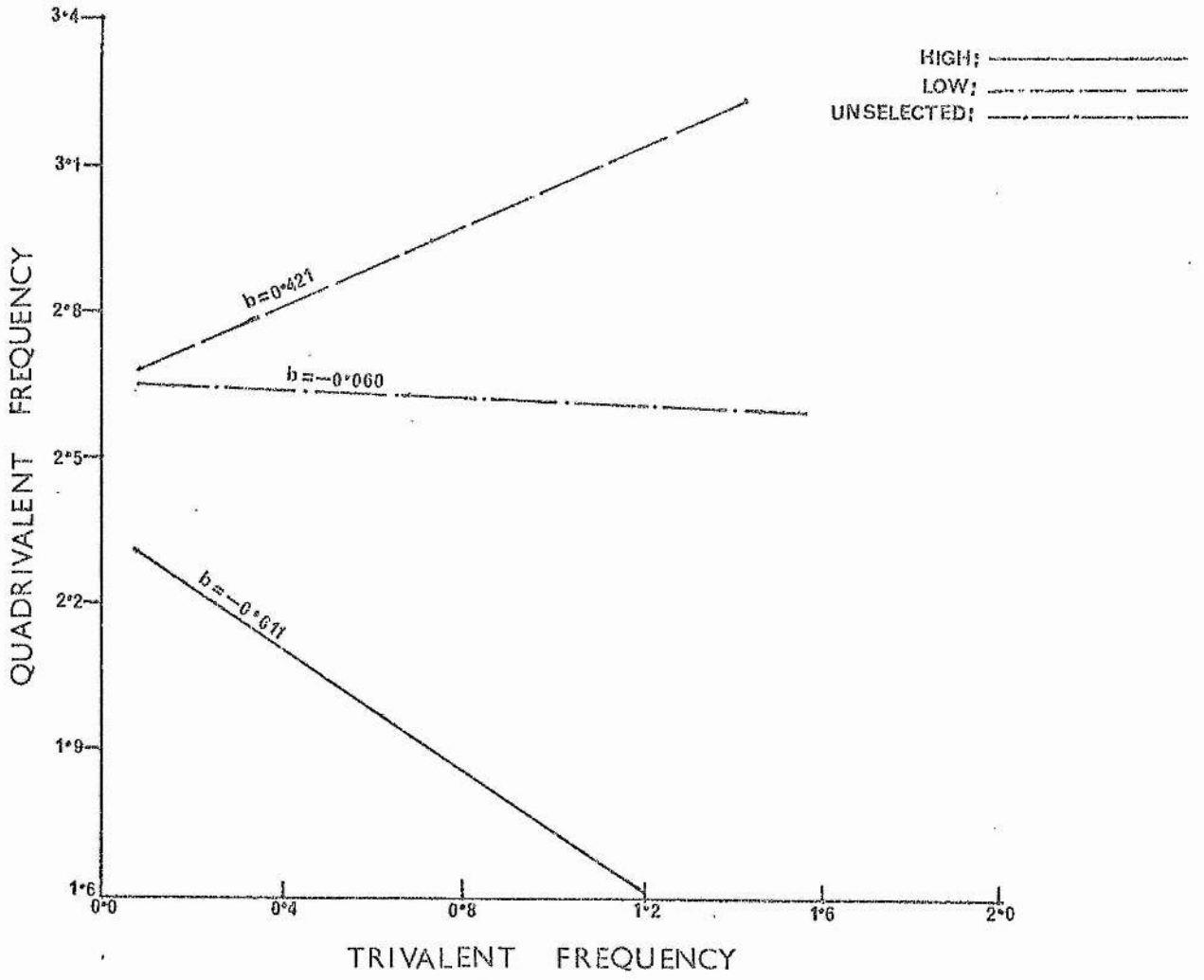


FIGURE II.13

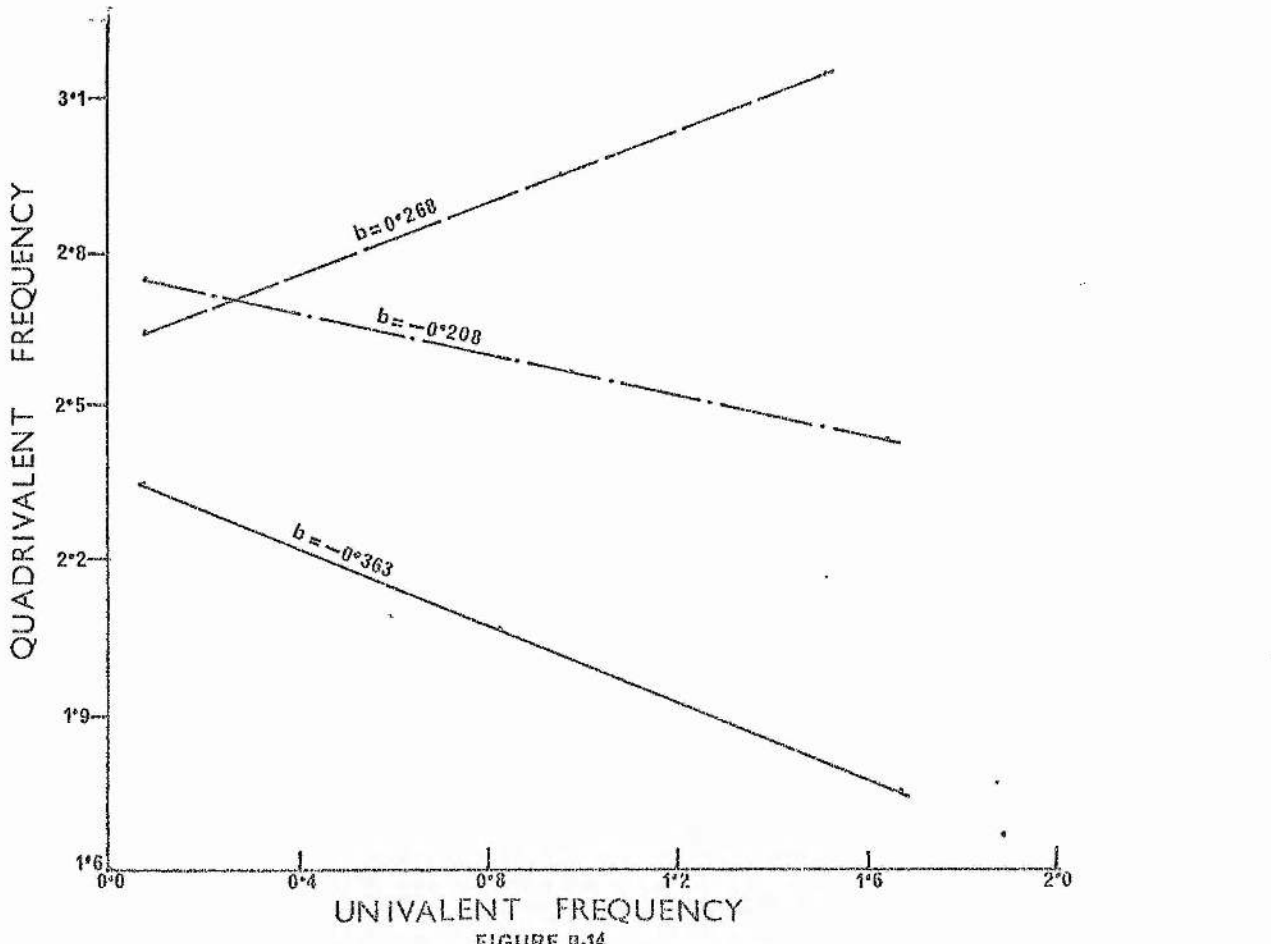


FIGURE II.14

Table II.24a. Variance Analyses for Regression of Quadrivalent Frequency on Trivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	0.70645	4.851*	1	0.06054	0.480 n.s.	1	0.00193	0.029 n.s.
Error	38	0.14562		21	0.12621		38	0.06700	

Table II.24b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.37516	0.18758	1.712 n.s.
Within Sample	97	10.62708	0.10956	
Total	99	11.00224		

* indicates significant at 5% level

n.s. " not significant

pairing in favour of bivalents, so that bivalents can be formed at the expense of trivalents but hardly a quadrivalent. This strengthens the view that chiasma distribution pattern in the unselected population is organised in such a way that it favours bivalent formations and not quadrivalents.

The positive correlation between quadrivalent and trivalent frequencies in the "low" population may seem surprising. We may recall here that in this population there are apparently some abnormal genotypes where, of the total seven sets of chromosomes, some sets have one homologue differentiated structurally or by genic mutation. In such a homologous set, the three homologous chromosomes will often be associated as a trivalent with favourable chiasma conditions, leaving the differentiated homologue as a univalent. Whereas in the rest of the seven sets, where the four homologues are identical, quadrivalent formations will be encouraged with favourable chiasma conditions. The result is a simultaneous increase in the frequencies of quadrivalents and trivalents as observed in figure II.13.

Now when one of the three normal homologues fails to pair with its normal counterparts, this provides a non-competitive chance for the differentiated homologue to undergo synapsis and thus forming a bivalent. As stated earlier, the number of chiasmata in such bivalents will be reduced because the differentiated segment (presumably one end) will

not take part in chromatid exchange. This seems to be the cause for the negative correlation between chiasma frequency and bivalent frequency observed earlier in figure II.10.

However, the heterogeneity between regression slopes is insignificant which suggests that the populations are not yet distinctly different in their chiasma distribution pattern, although diverging tendencies are evident.

Univalents

The relations between frequencies of quadrivalents and univalents in the three populations are demonstrated in figure II.14 and the separate regression analyses for the populations appear in table II.25a. The respective populations show, as expected, the same pattern of relationship as with trivalent frequency. Again the heterogeneity between regression slopes is insignificant.

Disjunction Index

Figure II.15 shows the relationship between quadrivalent frequency and disjunction index and the regression analyses are set out in table II.26a. The regression is positive and significant in the "high" population ($P < 0.01$). In the unselected population there is hardly any change in disjunction index as a result of changes in quadrivalent

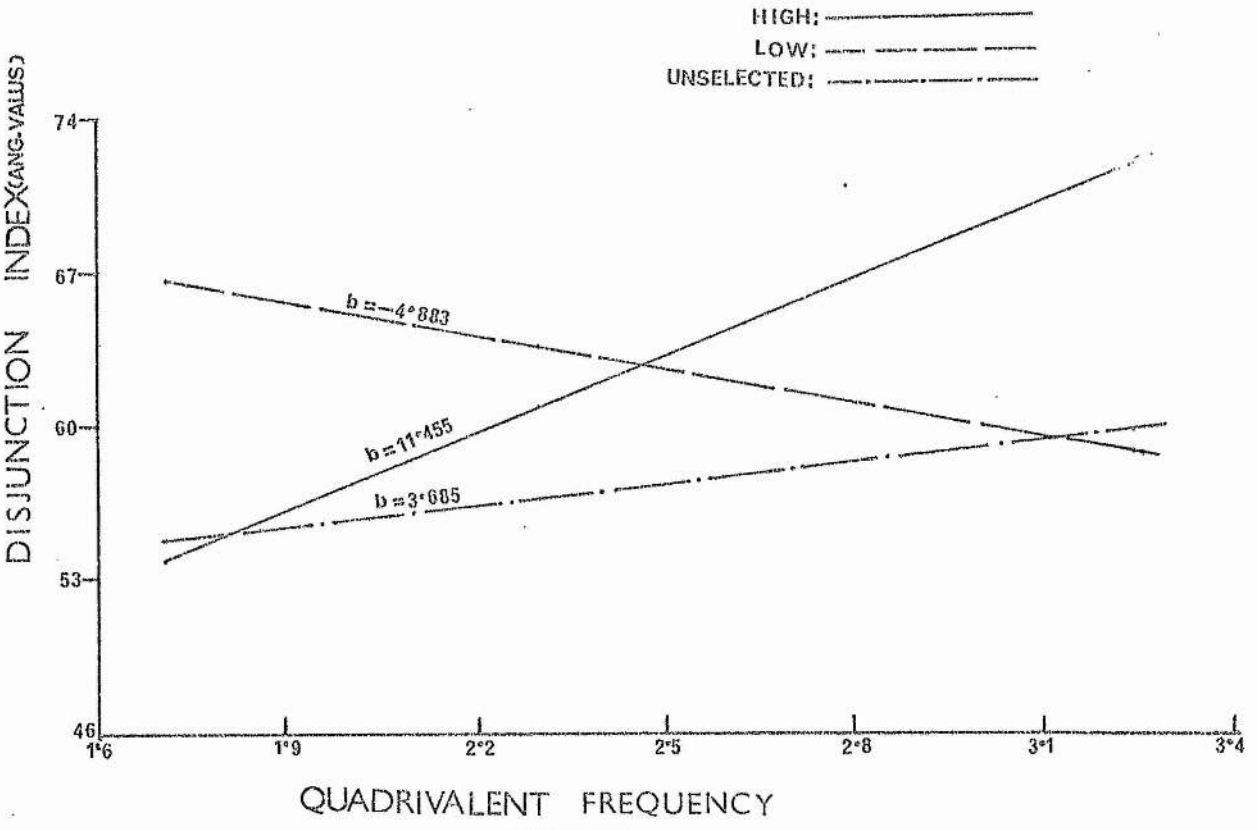


Figure II.15

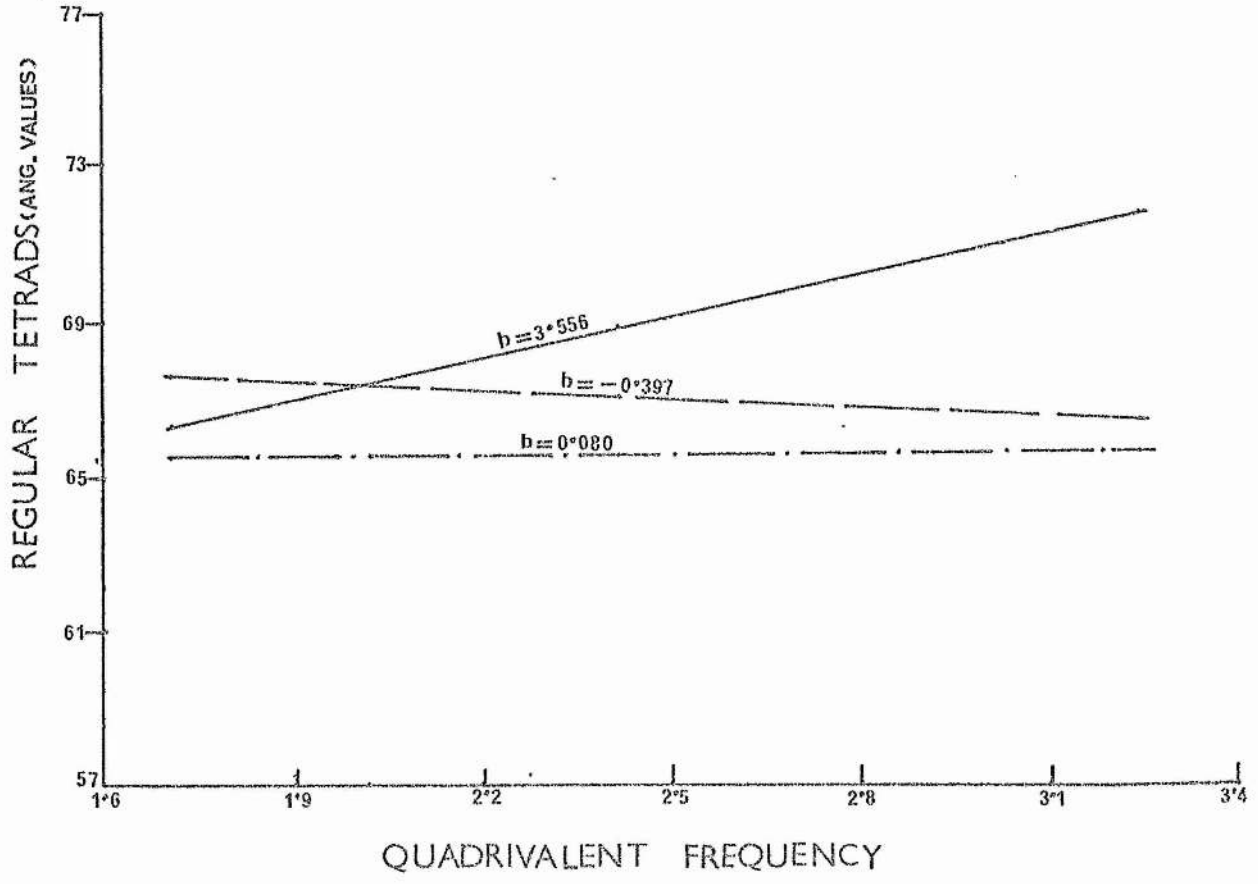


Figure II.16

Table II.25a. Variance Analyses of Regression of Quadrivalent Frequency on Univalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	0.63233	4.285*	1	0.06386	0.507 n.s.	1	0.10184	1.582 n.s.
Error	38	0.14757		21	0.12605		38	0.06437	

Table II.25b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.31880	0.15940	1.459 n.s.
Within Sample	97	10.59797	0.10926	
Total	99	10.91677		

* indicates significant at 5% level

n.s. " not significant

Table II.26a. Variance Analyses of Regression of Disjunction Index on Quadrivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	818.85344	7.533**	1	64.63660	0.692 n.s.	1	34.59707	0.468 n.s.
Error	38	108.69669		21	93.43681		38	73.96040	

Table II.26b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	516.69636	258.34818	2.815 n.s.
Within Sample	97	8903.14289	91.78498	
Total	99	9419.83925		

** indicates significant at 1% level

n.s. " not significant

frequency and the regression is insignificant. As before, the "low" population shows, which would not be normally expected, a negative relationship between disjunction index and quadrivalent frequency. From the positive correlation between quadrivalent frequency on one hand, and trivalents and univalents on the other (figures II.13 and II.14) this was expected because increases in the frequencies of trivalents and univalents always cause a decrease in disjunction index. Again the heterogeneity between regression slopes of the three populations is insignificant (table II.26b).

Regular Tetrads.

The relationship of quadrivalent frequency with regular tetrads in the three populations is illustrated in figure II.16 and the regression analyses appear in table II.27a. The relationship in the respective populations is essentially of the same trend as found with disjunction index. The regression is positive and significant in the "high" population and insignificant in the unselected and the "low" populations, in the latter the regression is negative. Once again the heterogeneity between regression slopes is insignificant.

Table II.27a. Variance Analyses of Regression of Regular Tetrads on Quadrivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	78.92715	3.233*	1	0.42726	0.034 n.s.	1	0.01628	0.001 n.s.
Error	38	24.41449		21	12.61941		38	25.46351	

Table II.27b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	40.16766	20.08383	0.907 n.s.
Within Sample	97	2148.74031	22.15196	
Total	99	2188.90797		

* indicates significant at 5% level

n.s. " not significant

c. Bivalents and Other Meiotic Features.

Trivalent

The relationships between the frequencies of bivalents and trivalents in the three populations are shown in figure II.17 and their variance analysis in table II.28a. The regressions are negative and significant at 5%, 1% and 0.5% levels in the "high, the "low" and the unselected populations respectively. Here again the heterogeneity between regression slopes is insignificant (table II.28b). Nevertheless, the differences in the levels of significance of the regressions point to the variable relationship between the two configurations. Of particular interest, the correlation is the highest in the unselected population which suggests that increased heterozygosity in this population imposes greater restriction in multivalent formations and favours disomic pairing.

It would be recalled that the regression slope between trivalents and quadrivalents was greater for the high population ($b = -0.611$) and the same between trivalents and bivalents is greater for the unselected population ($b = -3.029$). In other words, with decreasing trivalents, the rate of increase in quadrivalents is greater in the "high" population whereas in the "low" population the rate of increase is greater for bivalents. This also supports that chiasma distribution pattern in the unselected population favours disomic pairing.

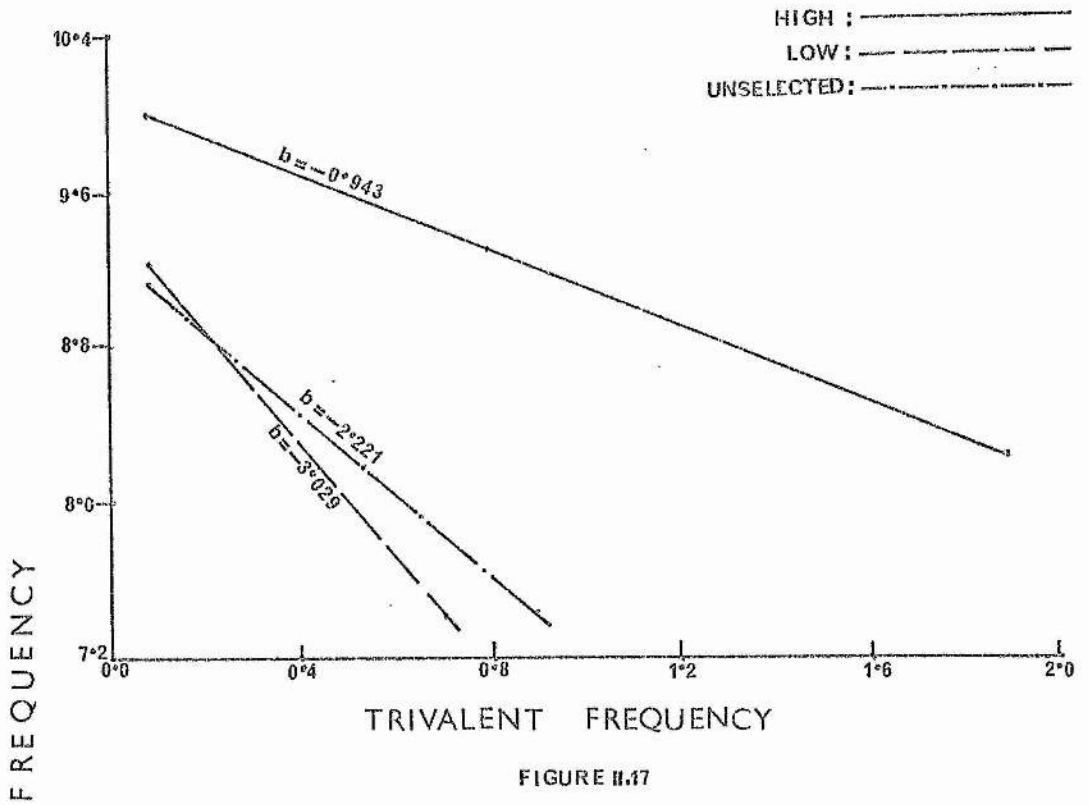


FIGURE II.17

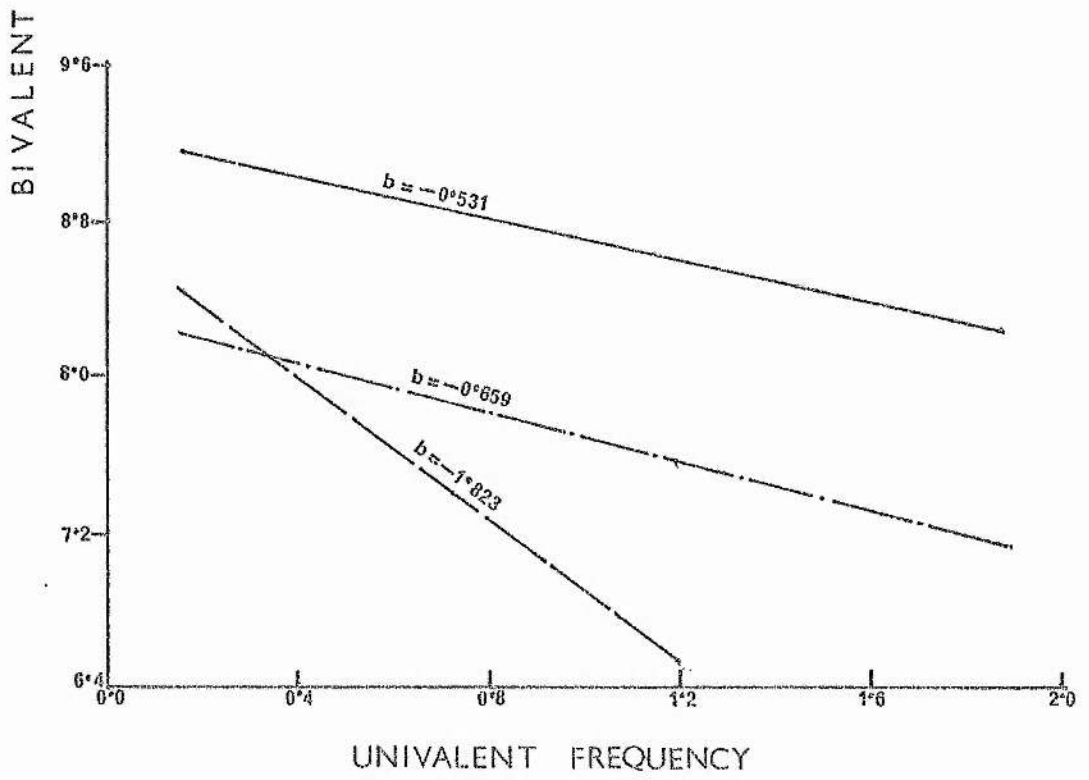


FIGURE II.18

Table II.28a. Variance Analyses of Regression of Bivalent Frequency on Trivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1.68115	2.915*	1	3.13007	6.117**	1	2.63945	10.534**
Error	38	0.57676		21	0.51173		38	0.25057	

Table II.28b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	1.65972	0.82986	1.908 n.s.
Within Sample	97	42.20033	0.43505	
Total	99	43.86005		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

Table II.29a. Variance Analyses of Regression of Bivalent Frequency on Univalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1.35155	2.309 n.s.	1	2.96428	5.705 *	1	1.01850	3.473 *
Error	38	0.58543		21	0.51962		38	0.29322	

Table II.29b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	0.83915	0.41958	0.918 n.s.
Within Sample	97	44.31667	0.45687	
Total	99	45.15582		

* indicates significant at 5% level

n.s. " not significant

Univalents

The regressions of bivalent frequency on the frequency of univalents in different populations are illustrated in figure II.18. Table II.29a gives the variance analyses for the regressions. Like trivalents, univalent frequency is negatively correlated with bivalent frequency in each of the three populations. The regression, however, is insignificant in the "high" as well as in the unselected populations and significant only in the case of the "low" population ($P < 0.05$). The coefficient of regression in the latter ($b = -1.823$) is about twice as much as that of the former two populations. It is, therefore, apparent that in the "low" population there is a higher rate of failure in bivalent formation, possibly because of the abnormal chromosome complement suggested earlier, whereas with normal chromosome complement bivalent formation is much more rigid in the "high" and the unselected populations. In other words, there are very few univalents in the "high" and the unselected populations that arise from the failure of bivalent formation. Unfortunately the heterogeneity between regression slopes is insignificant. (table II.29b).

Disjunction Index

As expected, disjunction index is positively regressed on bivalent frequency in each of the three populations (figure II.19). The regression is significant at 1% and 5%

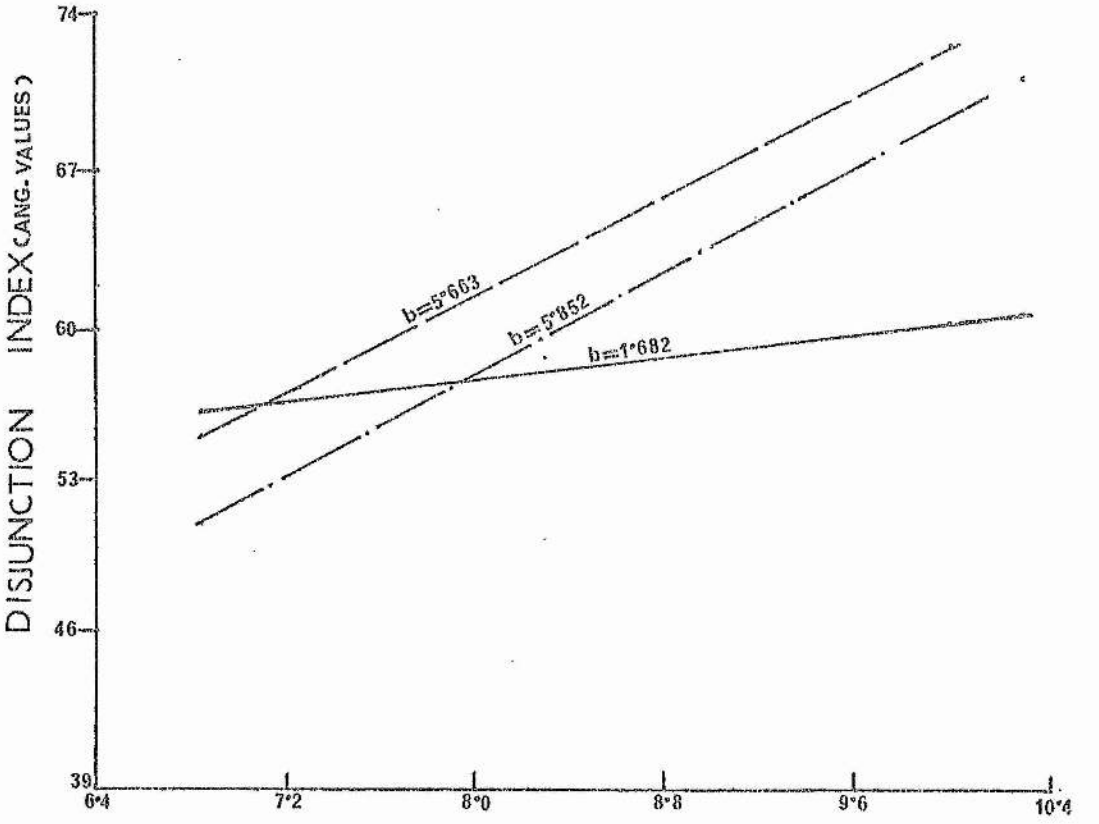


Figure II.19

HIGH: ——————
LOW: — — — — —
UNSELECTED: —·—·—·—

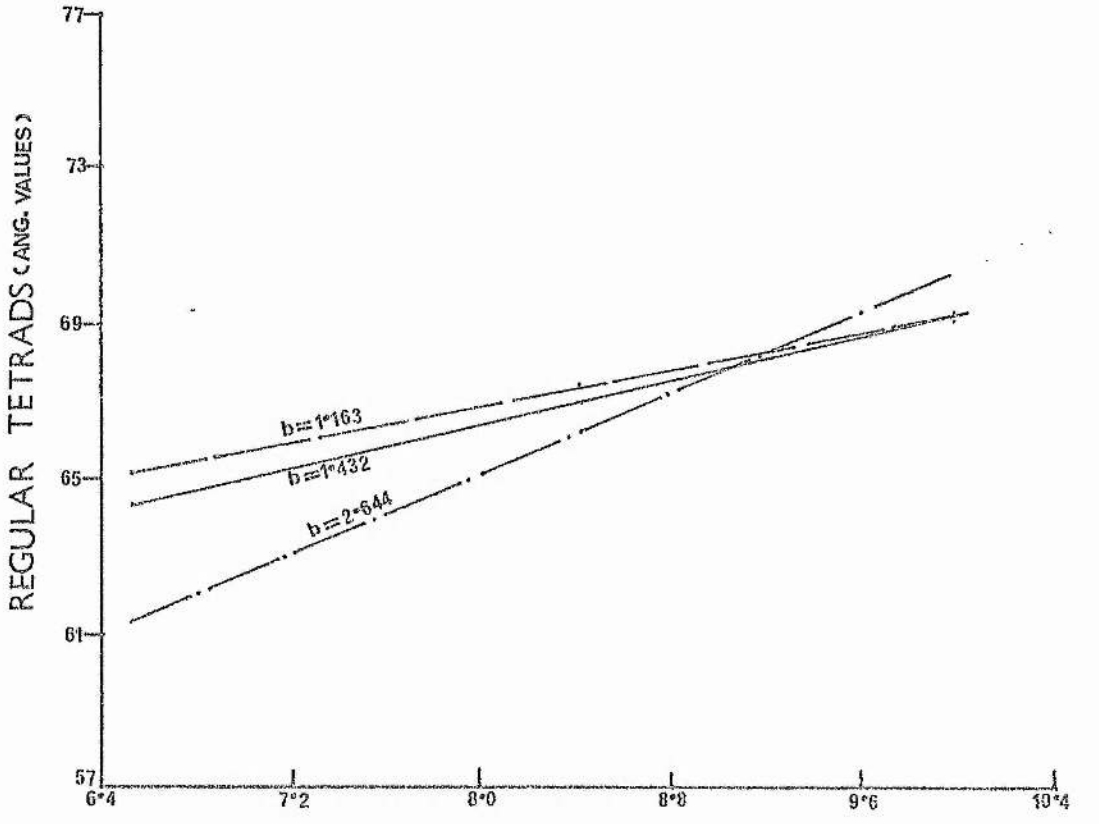


Figure II.20

Table II.30a. Variance Analyses of Regression of Disjunction/Index on Bivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	66.79269	n.s. 0.520	1	445.06421	* 5.909	1	416.47342	** 6.516
Error	38	128.4877		21	75.32121		38	63.91103	

Table II.30b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	205.42199	102.7100	n.s. 1.120
Within Sample	97	8892.89968	91.67938	
Total	99	9098.32167		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

levels in the unselected and the "low" populations respectively (table II.30a). In the "high" population the regression is insignificant. This was expected from the higher correlation between quadrivalent frequency and disjunction index observed earlier for the "high" population (figure II.15 and table II.26a). Because both quadrivalents and bivalents contribute to disjunction index and an increase in the rate of contribution of one reduces the rate of contribution of the other. This is more so, because the frequencies of quadrivalents are negatively correlated with the frequencies of bivalents (appendix table 3). By the same token, if quadrivalent frequency is not significantly correlated with disjunction index, we would expect bivalents to contribute significantly. This is demonstrated by both the "low" and the unselected populations. Again the heterogeneity of regression is not significant suggesting that the populations are not distinct enough in respect of pairing pattern.

Regular Tetrads

As observed with disjunction index, the relationship between bivalent frequency and the proportions of regular tetrads is positive in each population (figure II.20). The regression is significant only in the unselected population ($P = 0.05$, table II.31a). This confirms that disomic pairing dominates the chromosome association pattern in the unselected population. Again the heterogeneity between regression slopes of the populations is insignificant.

Table II.31a. Variance Analyses of Regression of Regular Tetrads on Bivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	48.38641	n.s. 1.919	1	18.76626	n.s. 1.598	1	84.98793	3.659 *
Error	38	25.21819		21	11.74612		38	23.22741	

Table II.31b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	16.46210	8.23105	n.s. 0.385
Within Sample	97	2075.97040	21.40176	
Total	99	2092.43250		

* indicates significant at 5% level

n.s. " not significant

IV. Seed-Set and Meiotic Features

a. Bivariate Analyses

As stated earlier, although there are good reasons for supposing that the fertility in autotetraploids is affected by meiotic chromosome behaviour, there are conflicting reports as to the relationship between the degree of meiotic irregularities and the level of seed-set. In inbred materials the existence of the correlation has been well demonstrated (Roseweir & Rees, 1962; Hazarika & Rees, 1967). At the same time these authors have pointed out that the relationship is not true for all genotypes, some inbred lines behave exceptionally depending on the influence exerted by "other factors" (Hazarika & Rees, 1967). According to many workers these are physiological factors. The following analyses were made firstly, to distinguish between populations with respect to the relationship between seed-set and meiotic behaviour and, secondly, to understand the nature of interaction, if any, between the so-called physiological factors and the cytological factors.

Chiasma Frequency

Figure II.21 shows the regression of seed-set on chiasma frequency in the "high", the "low" and the unselected populations. Their variance analyses are presented in table II.32a. If

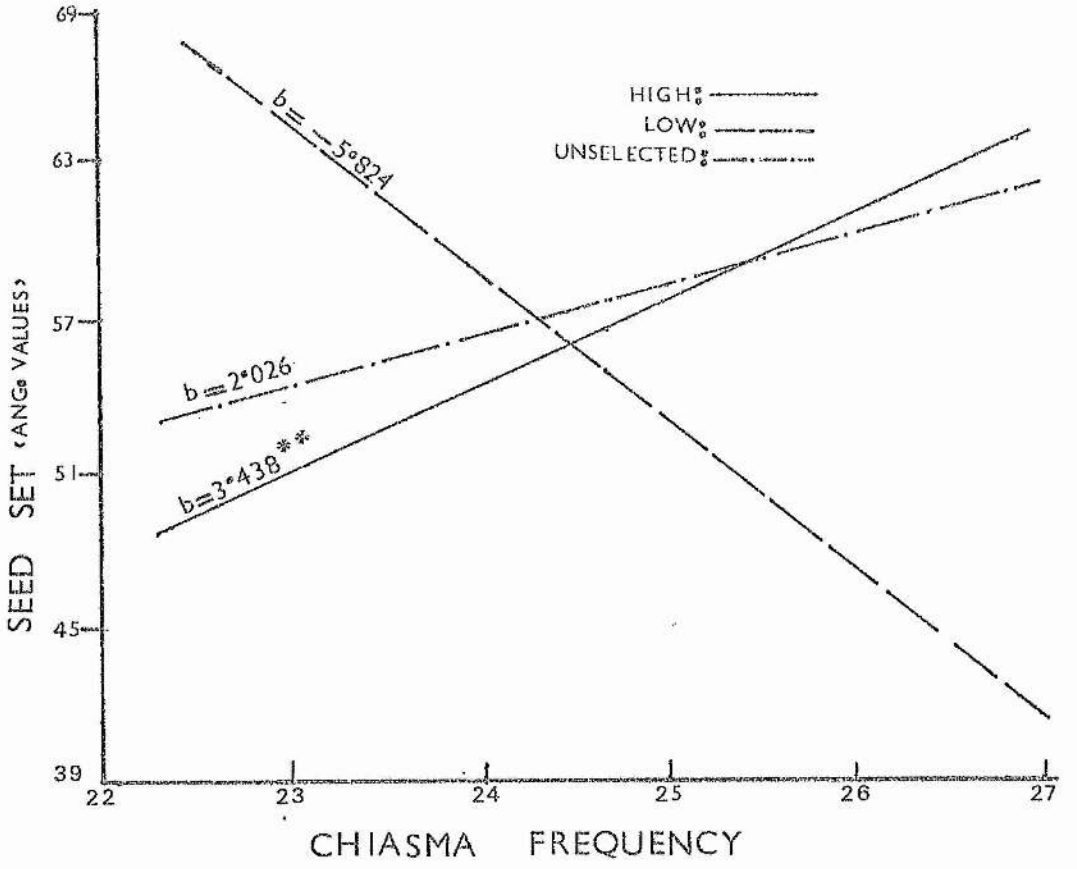


FIG. II.21

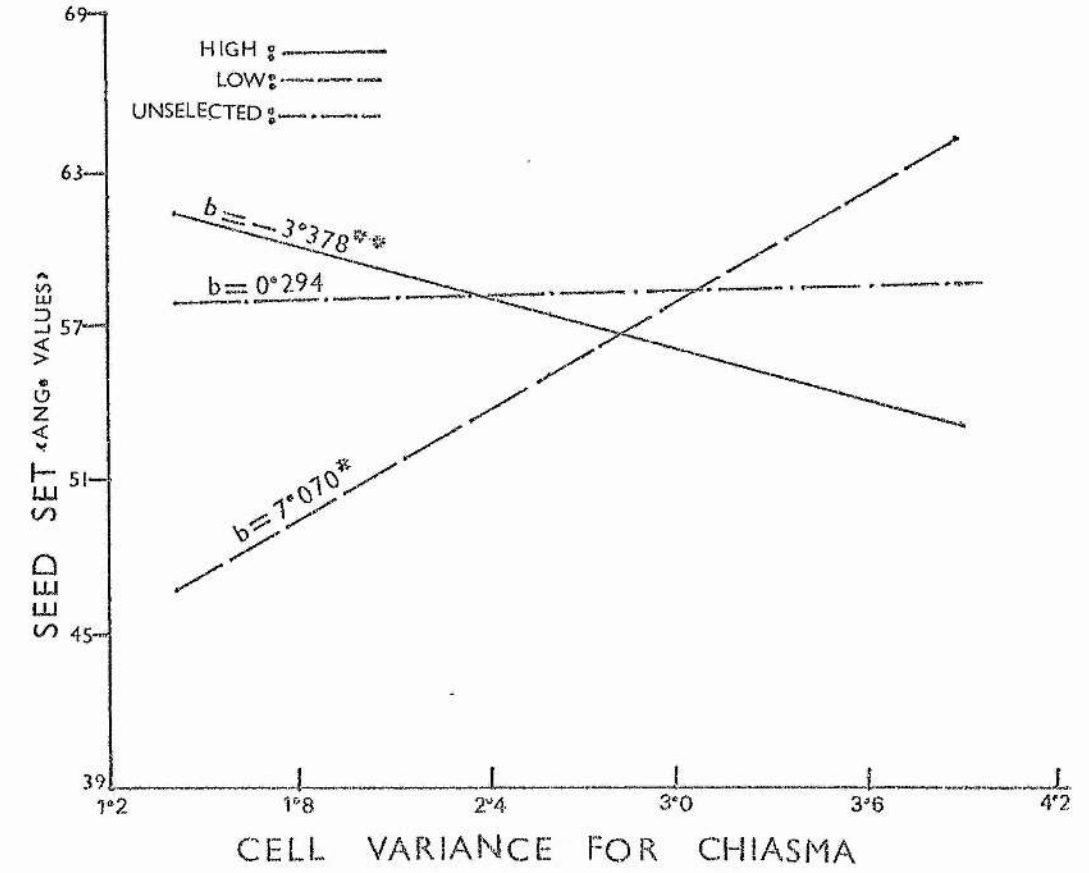


FIG. II.22

Table II.32a. Variance Analyses of Regression of Seed-Set on Chiasma Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	752.56293	10.179**	1	218.88654	1.572 n.s.	1	100.89334	3.067
Error	38	73.93118		21	139.21100		38	32.89333	

Table II.32b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	509.7098	254.8549	3.5403*
Within Sample	97	6982.8153	71.9878	
Total	99	7492.5251		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

chiasma frequency affects seed-set, we would expect a positive correlation between the two characters. This is, in fact, demonstrated by the "high" population ($b=3.438$, $p<0.01$), but in the unselected population the regression is below 5% level of significance. In the "low" population, the regression is not only insignificant but also negative in sign. Also the heterogeneity test for regression slopes is significant ($P<0.05$) suggesting that the dependence of seed-set on chiasma frequency does vary between populations (table II.32a).

Cell-Variance for Chiasmata

The relationships between cell-variance and seed-set in the three populations are illustrated in figure II.22 and the regression analyses appear in table II.33a. Cell-variance gives a measure of irregular chromosome association so that the higher the variance, the lower is the pairing regularity. One would, therefore, expect a negative correlation between cell variance and seed-set. This is observed in the "high" population where the regression is highly significant ($P<0.01$). In contrast the regression is positive and significant in the "low" population ($P<0.05$). This would not be expected if pairing behaviour had a direct influence on fertility in this population. An intermediate and insignificant relationship is observed with the unselected population. The heterogeneity between regression slopes is also highly significant ($P<0.01$) (table II.33b). This again suggests that the dependence of seed-set on chromosome behaviour can vary between populations.

Table II.33a. Variance Analyses of Regression of Seed-Set on Cell-Variance for Chiasmata in High, Low and Unselected Populations, 1972

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regressions	1	587.08899	7.499**	1	502.26759	3.995*	1	2.65992	n.s. 0.075
Error	38	78.28575		21	125.71666		38	35.47842	

Table II.33b. Variance Analysis of Heterogeneity of Regressions between Populations

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	1000.1419	500.0710	6.966**
Within Sample	97	6963.1415	71.7850	
Total	99	7963.2834		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

Quadrivalent Frequency

The regression of seed-set on quadrivalent frequency is shown in figure II.23 and the variance analyses in table II.34a. Unlike the findings in inbred lines (Hazarika & Rees, 1967), there is no significant relationship in any populations, nor is the heterogeneity between regression slopes significant (table II.34b). It is interesting to note that in the unselected population, the regression is negative. It may be recalled that in this population, the correlations of chiasma frequency with different meiotic features revealed a preference for disomic pairing. Since it is apparent that quadrivalent frequency increases with increasing homozygosity (Ellis, et al, 1973; MacKey, 1970), while the latter causes inbreeding depression, it is not surprising that with increased quadrivalent frequency there is a decrease in seed-set in this random mating population.

Bivalent Frequency

Like quadrivalents, the frequencies of bivalents do not show any significant relationship with seed-set (see figure II.24 and table II.35a and b).

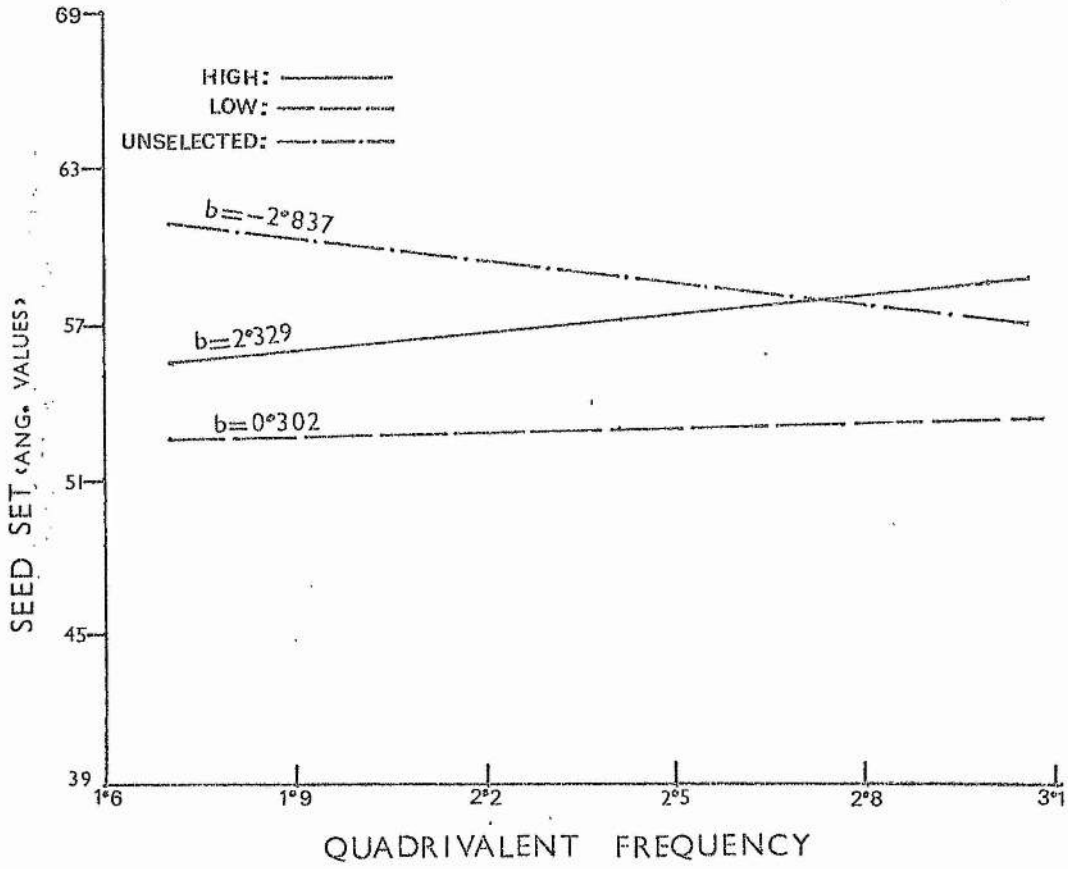


FIG. II.23

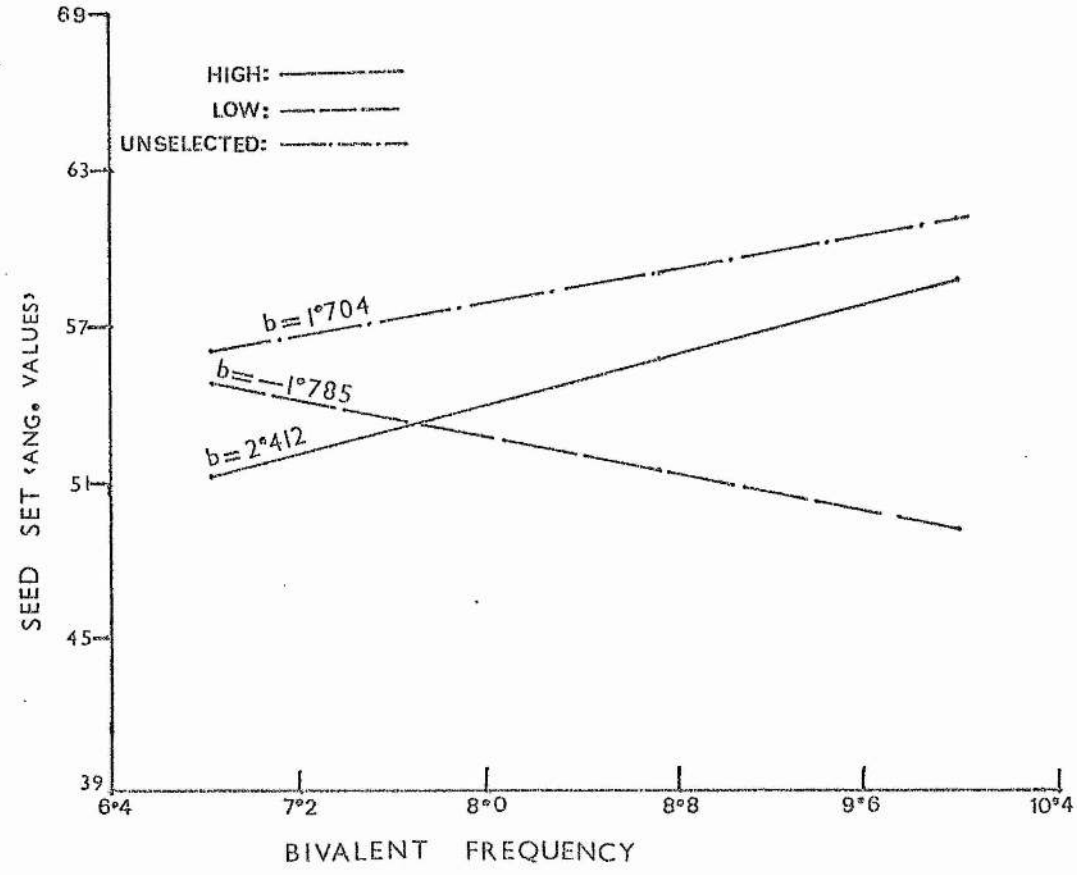


FIG. II.24

Table II.34a. Variance Analyses of Regression of Seed-Set on Quadrivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	33.85277	0.365 n.s.	1	0.24812	0.002 n.s.	1	20.51396	0.586 n.s.
Error	38	92.84460		21	149.62235		38	35.00857	

Table II.34b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	48.8218	24.4109	0.296 n.s.
Within Sample	97	8000.5431	82.4798	
Total	99	8048.3649		

n.s. indicates not significant

Table II.35a. Variance Analyses of Regression of Seed-Set on Bivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	137.32471	n.s. 1.524	1	44.23301	n.s. 0.306	1	35.28877	n.s. 1.019
Error	38	90.12166		21	147.52783		38	34.61976	

Table II.35b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	160.5527	80.2764	n.s. 0.993
Within Sample	97	7838.3115	80.8073	
Total	99	7998.8642		

n.s. indicates not significant

Number of Chromosomes Involved in (IV + II) Formations

From the above results, it is evident that in the present materials the frequencies of quadrivalents and bivalents, when considered separately, do not have any significant effect on seed-set. Since both these configurations contribute to the formation of balanced gametes by equal chromosome separation at anaphase-I, the total number of chromosomes involved in them would normally be expected to be positively correlated with seed-set. The relationships between the number of chromosomes in IVs + IIs formations and seed-set in the three populations have been shown in figure II.25 and the regression analyses are given in table II.36a. As expected, the regression is positive and significant in the "high" population ($P < 0.01$). But in the "low" population the regression is negative and an intermediate relationship is found in the unselected population. This supports the earlier conclusion that the relationship between pairing configurations and seed-set can be variable and this is confirmed by the significant heterogeneity between regression slopes ($P = 0.05$) (Table II.36b).

Trivalent Frequency

Seed-set is adversely affected with increasing trivalent frequency in both the "high" and the unselected populations (figure II.26). This would be expected with normal chromosome complement. The variance analysis in table II.37a

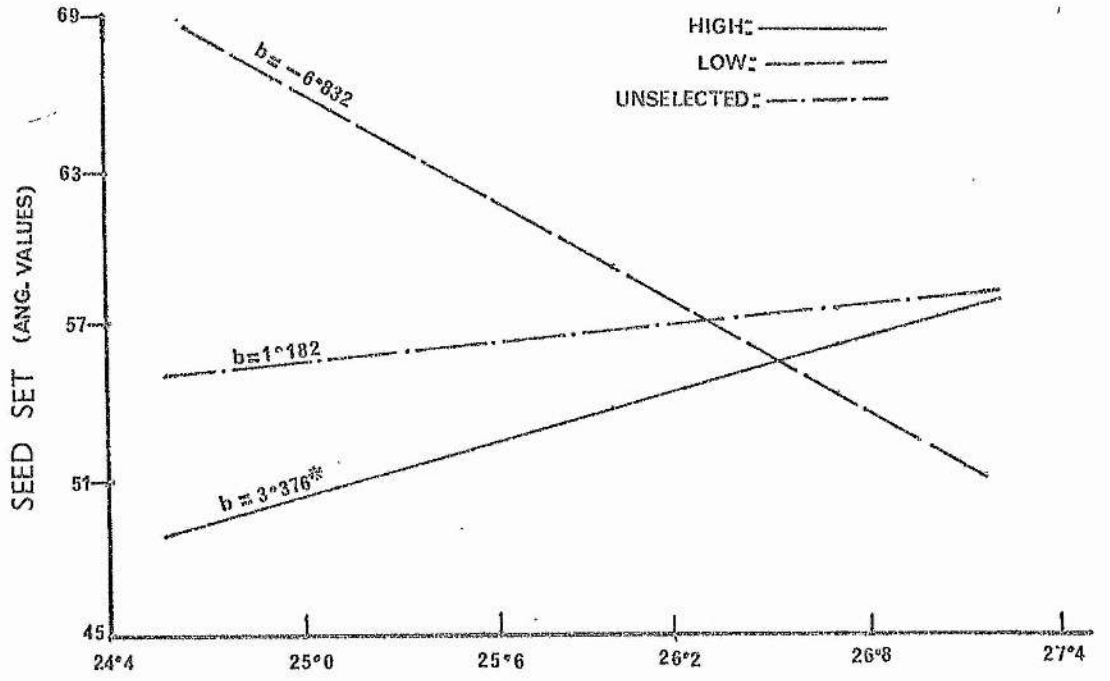

HIGH:
LOW:
UNSELECTED:
b= -6°832
b=1°182
b= 3°376*
SEED SET (ANG. VALUES)
69
63
57
51
45
24°4
25°0
25°6
26°2
26°8
27°4
NUMBER OF CHROMOSOMES IN (IV+II) FORMATIONS

FIG. II.25

Table II.36a. Variance Analyses of Regression of Seed-Set on Number of Chromosomes Involved in (IVs + IIs) Formations in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	420.38899	5.085**	1	316.11319	2.349 n.s.	1	18.55637	0.529 n.s.
Error	38	82.67260		21	134.58116		38	35.06009	

Table II.36b. Variance Analysis of Heterogeneity of Regressions between Populations

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	599.99760	299.99880	3.986*
Within Sample	97	7300.09945	75.25876	
Total	99	7900.09705		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

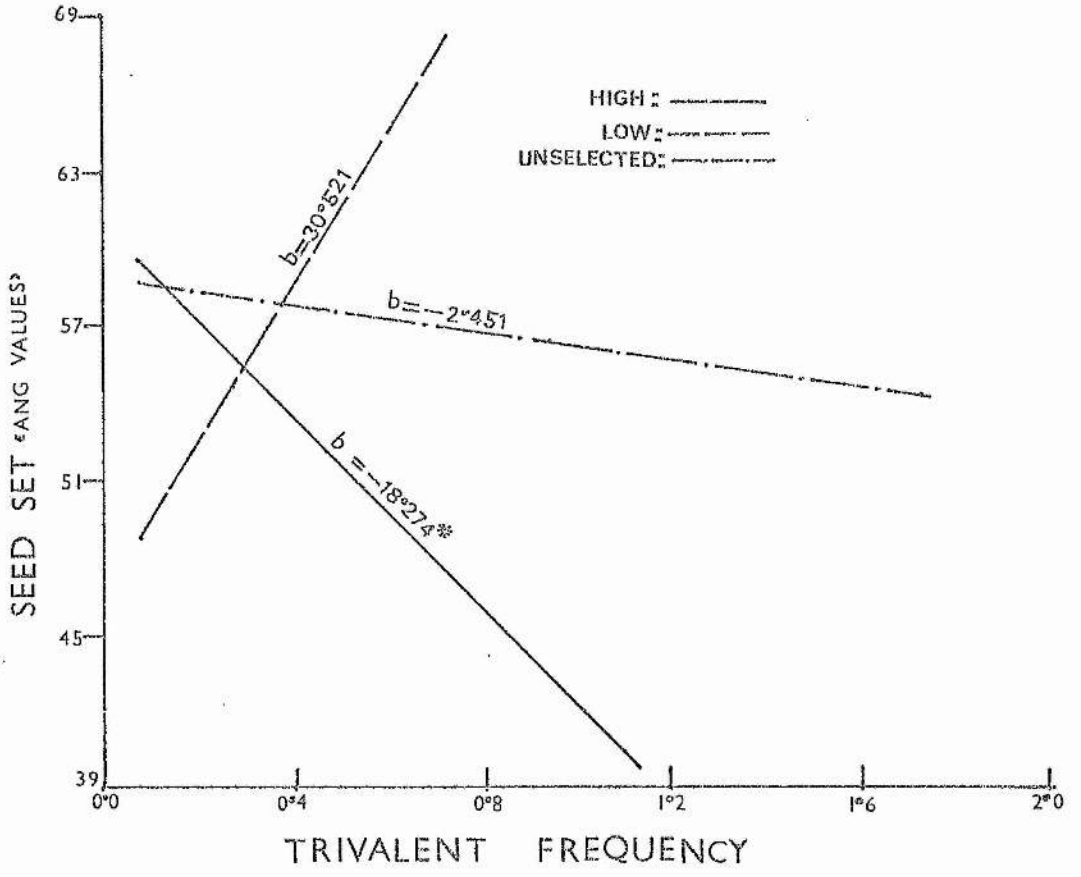


FIG. II.26

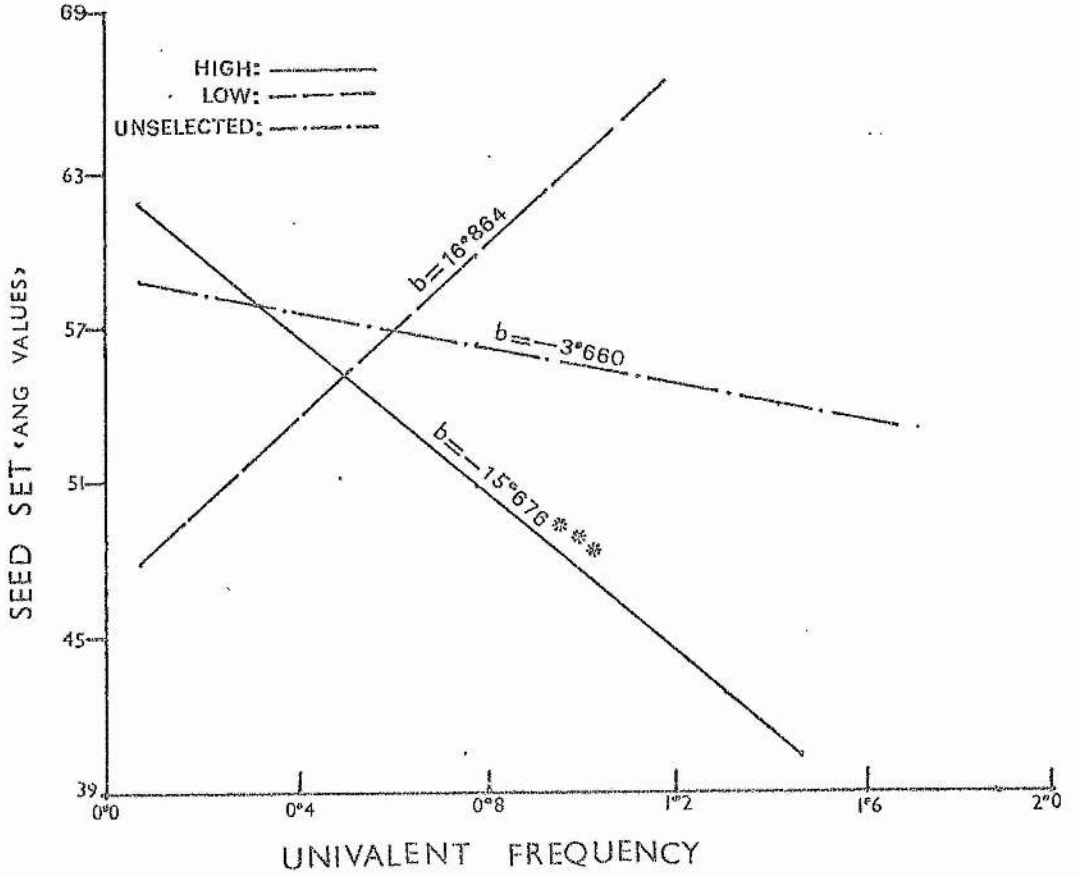


FIG. II.27

show that the regression is significant in the "high" population only ($P < 0.05$). In the "low" population, however, the regression is positive but insignificant. The heterogeneity of regression slopes is also highly significant ($P < 0.01$ in table II.37b), which again suggests that the dependence of seed-set on trivalent frequency can be variable.

Univalent Frequency

As expected, seed-set shows the same pattern of dependence on univalent frequency as observed with trivalents in the respective populations. That is, the regression is negative in both the "high" and the unselected populations and positive in the "low" population (figure II.27). Again the regression is significant only in the "high" population ($P < 0.001$, table II.38a). The heterogeneity between regression slopes is also highly significant ($P < 0.01$, table II.38b).

Disjunction Index

Disjunction index gives an estimate of the proportion of balanced gametes that would be expected from the chromosome pairing pattern at meiosis. It is, therefore, expected that seed-set would be correlated with disjunction index. The relation between these two characters in the three populations have been shown in figure II.28 and table II.39a presents their variance analyses. In the "high" population the

Table II.37a. Variance Analyses of Regression of Seed-Set on Trivalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	631.45113	8.188**	1	317.74192	2.362 n.s.	1	3.21422	0.091 n.s.
Error	38	77.11833		21	134.50360		38	35.46383	

Table II.37b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	718.4204	359.2102	4.906**
Within Sample	97	7102.7508	73.2242	
Total	99	7821.1712		

** indicates significant at 1% level

n.s. " not significant

Table II.38a. Variance Analyses of Regression of Seed-Set on Univalent Frequency in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	1180.36376	18.834***	1	253.59050	1.844 n.s.	1	31.40200	0.904 n.s.
Error	38	62.67362		21	137.55843		38	34.72205	

Table II.38b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	893.4733	446.7367	6.5788**
Within Sample	97	6589.8017	67.9361	
Total	99	7483.2750		

** indicates significant at 1% level

*** indicates " at 0.1% level

n.s. " not significant

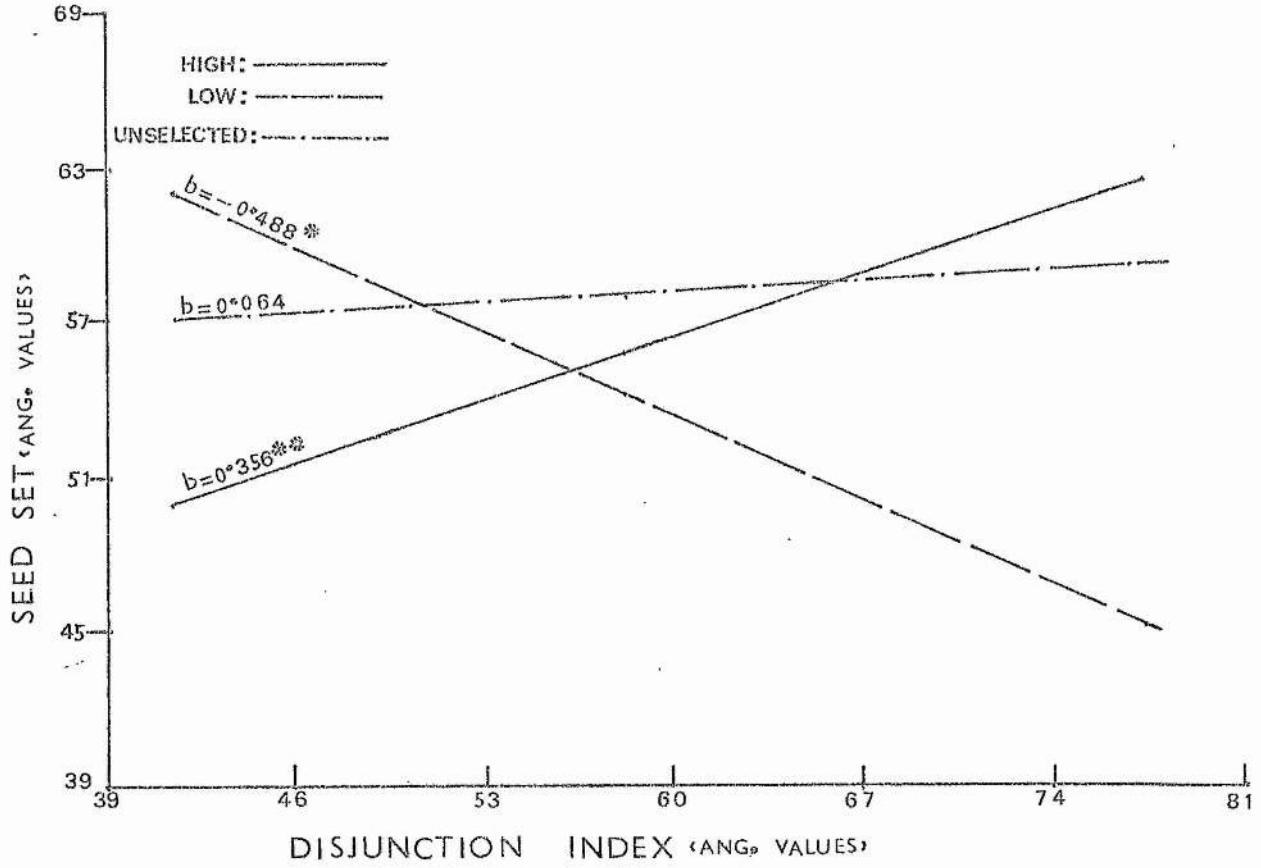


FIG. II.28

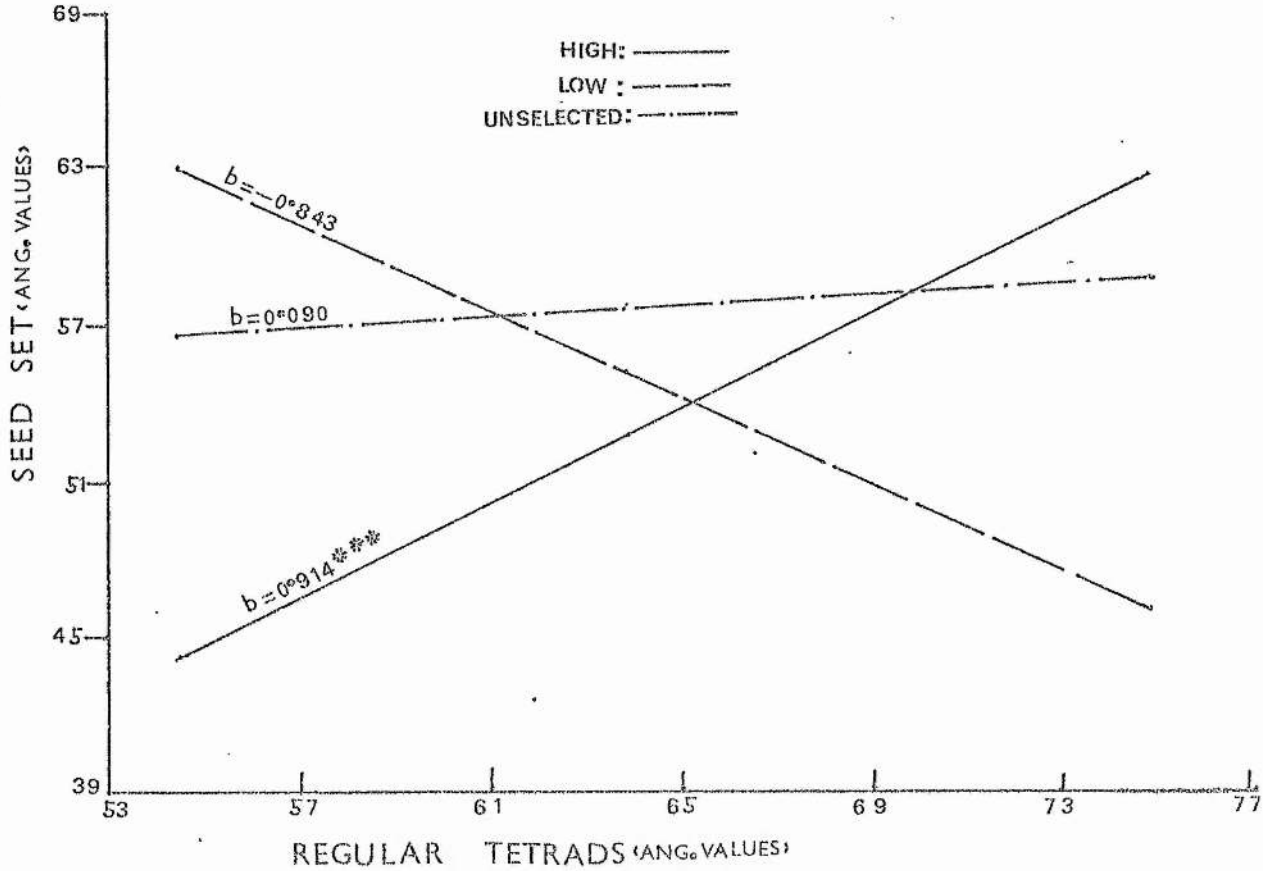


FIG. II.29

Table II.39a. Variance Analyses of Regression of Seed-Set on Disjunction Index in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	627.62612	8.128**	1	483.03743	3.814*	1	11.71329	0.332 n.s.
Error	38	77.21899		21	126.63238		38	35.24017	

Table II.39b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	1029.3972	514.6986	7.201**
Within Sample	97	6932.7812	71.4720	
Total	99	7962.1784		

* indicates significant at 5% level

** " " at 1% level

n.s. " not significant

regression is positive and significant ($P < 0.01$). In the "low" population, however, the regression is negative and also significant ($P < 0.05$). The regression line of the unselected population lies in between those of the other two populations. The regression heterogeneity is again highly significant ($P < 0.01$, table II.39b). Thus, like individual configuration types, disjunction index also shows variable effects on fertility. This was indeed expected because disjunction index is directly obtained from configuration types.

Regular Tetrads

Figure II.29 shows the regression of seed-set on the proportion of regular tetrads for the three populations. The analyses of variance for the regressions are presented in table II.40a. The results for the respective populations are in good agreement with those found with disjunction index. That is, there is a positive and significant regression in the "high" population ($P < 0.001$), positive but insignificant in the unselected population and an insignificant negative regression in the "low" population. The heterogeneity of regression slopes is also significant to the same extent ($P < 0.01$, table II.40b), as with disjunction index.

Table II.40a. Variance Analyses of Regression of Seed-Set on Regular Tetrads in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	841.30593	11.751**	1	188.78002	1.342 n.s.	1	7.91184	0.224 n.s.
Error	38	71.59583		21	140.64464		38	35.34021	

Table II.40b. Variance Analysis of Heterogeneity of Regressions between Populations.

ITEMS	D.F.	S.S.	M.S.	F
Between Regressions	2	762.2093	381.1047	5.268**
Within Sample	97	7017.1603	72.3419	
Total	99	7779.3696		

** indicates significant at 1% level

*** " " at 0.1% level

n.s. " not significant

b. Multivariate Analyses.

The bivariate analyses provided evidence that the three populations differed significantly with respect to the influence of cytological factors on seed-set. This can be further tested by multivariate analysis if several cytological features affecting fertility can be regarded as independent variables. In the sets of data in the appendix (appendix tables 1A, 1B & 1C) for the three populations, (i) chiasma frequency, (ii) disjuction index and (iii) regular tetrads are cytological features representing the degree of meiotic regularity and satisfy conditions for independent variables of a multiple regression analysis. Such analyses for the three populations are set out in table II.41.

Table II.41a. Variance Analyses of Multiple Regression of Seed-Set on Chiasma Frequency, Disjunction Index and Regular Tetrads in High, Low and Unselected Populations, 1972.

ITEMS	HIGH			LOW			UNSELECTED		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Multiple Regression	3	299.79076	4.053*	3	182.04424	n.s. 1.332	3	49.99393	n.s. 1.297
Error	36	73.96043		19	136.64361		36	38.54008	
	R^2 = 25.25%			R^2 = 17.38%			R^2 = 9.76%		

The multiple regression is significant only in the "high" population ($P < 0.05$) and the meiotic features included in the regression account for 25.25% ($=R^2$) of the variability in seed-set in this population. In both "low" and unselected populations the multiple regression is insignificant and the proportions of variability in seed-set accounted for by the meiotic features (R^2) are only 17.38% and 9.76% respectively. From this it is further evident that cytological factors have variable influence on fertility in the three populations. This will be more clear from the normalised regression coefficients (β) of the three cytological features introduced in the multiple regression. The normalised regression coefficients (β) with corresponding regression coefficients (b) of the three independent variables in the multiple regression are set out in table II.41b.

Table II.41b. Regression Coefficients (b) and Normalised Regression Coefficients (β) of the Independent Variables Introduced in the Multiple Regression

INDEPENDENT VARIABLES	HIGH		LOW		UNSELECTED	
	b	β	b	β	b	β
1. Chiasma Frequency	+1.677	+0.224	+1.813	+0.082	+2.856	+0.361
2. Disjunction Index	-0.134	-0.142	-0.627	-0.509	+0.020	+0.027
3. Regular Tetrads	+0.721	+0.383	+0.192	+0.056	-0.148	-0.117

It will be seen from the table (II.41b) that of the three meiotic features, regular tetrads exerts the greatest influence on seed-set in the "high" population with a positive effect (β = +0.383); in the "low" population disjunction index has the greatest influence but with a negative effect (β = -0.509) whereas in the unselected population chiasma frequency influences seed-set more (β = +0.361) than the other two features.

The lack of a significant relationship between meiotic regularity and seed-set in the unselected and the negative relationship observed in the "low" population suggest that fertility can, at least to some extent, be independent of cytological factors. When this happens some other factors must take over the control of fertility. As many workers have already suggested that these "other factors" are physiological, the conclusion one can draw from the above results is that both cytological and physiological factors influence fertility and the relative importance of one over the other can vary between populations. Since the three populations investigated here were derived from the same original stock and subjected to varied influence of selection, it can also be concluded that the relative importance of cytological factors over the physiological ones and vice versa can be manipulated by selection pressure.

Unfortunately not much information is available in the literature about the physiological factors nor the way they

interact with cytological factors in determining fertility. On the face of it, one can consider that plant vigour, as measured by plant height, number of tillers, number of spikelets per spike, spike length and seed-set together would give an estimate of the physiological status of the plant. Having considered this one can attempt to find if there is any relationship between plant vigour and cytological features. This was done separately for the three populations by canonical correlation analyses. The first set of variables in the canonical correlations included three meiotic features namely, (i) chiasma frequency, (ii) disjunction index and (iii) regular tetrads while the second set of variables was composed of five morphological characters representing plant vigour. These were, as mentioned above, (i) plant height, (ii) number of tillers per plant, (iii) number of spikelets per spike, (iv) spike length and (v) seed-set. The summary results of canonical correlation analyses in the three populations are presented in tables II.42a & b.

The canonical correlation is significant only in the unselected population (P = 2%-5%). It will be recalled that under bivariate analyses none of the cytological features nor any of the morphological characters were significantly correlated with seed-set in this population. The significant canonical correlation probably suggests that the two sets of characters, cytological and physiological, supplement each other, i.e. when the cytological factors fall short, the physiological factors

Table II.42a. First Canonical Correlation between Meiotic Features and Morphological Characters in High, Low and Unselected Populations, 1972.

Population	Eigenvalue	Canonical Correlation	Wilk's Lambda	Chi-square	D.F.	P
HIGH	0.25795	0.50789	0.60624	17.76704	15	20%-30%
LOW	0.47540	0.68949	0.30524	21.95312	15	10%-20%
UNSELECTED	0.34844	0.59029	0.49336	25.08135	15	2%-5%

Table II.42b. Coefficients of First Canonical Correlation between Three Cytological Features and Five Morphological Characters in High, Low and Unselected Populations.

CHARACTERS	HIGH	LOW	UNSELECTED
I. Cytological Features			
Chiasma Frequency	+0.099	+0.086	-1.264
Disjunction Index	-0.242	+0.075	-0.061
Regular Tetrads	+1.056	+0.899	+0.618
II. Morphological Characters			
Plant Height	-0.062	-0.025	+0.579
No. of Tillers	-0.212	+0.199	+0.120
No. of Spikelets	-0.209	-0.748	-0.081
Spike Length	+0.403	-0.323	+0.537
Seed-Set	+0.905	-0.379	-0.570

come to the aid of seed-set in this population. If this is so, we would expect that the features of meiotic regularity would be negatively correlated with morphological characters but seed-set would be positively correlated with both meiotic features and morphological characters. These correlations are set out in table II.43.

As expected, the cytological features are negatively correlated with the morphological characters with a few exceptions (encircled) and seed-set is positively correlated with each of the cytological features as well as the morphological characters. It can, therefore, be concluded that in general, the so called physiological factors supplement the cytological factors in determining fertility in this unselected population.

The weak canonical correlation (table II.42a) and also the weak simple correlations between cytological features and morphological characters (table II.43) suggest that the two sets of factors may not be genetically linked. If this is so, the canonical correlation observed in the unselected population will vary in magnitude and become insignificant with appropriate selection pressure. At the same time it should be possible to reverse the negative correlations between cytological features and morphological characters to positive ones.

In the "high" population, plants were selected for both meiotic regularity and seed-set, the latter helped selection

Table II.43. Simple Correlation Coefficients between Cytological Features and Morphological Characters in the Unselected Population.

Morphological Characters	Cytological Features			Seed-set
	Chiasma Frequency	Disjunction Index	Regular Tetrads	
Plant Height	-0.374*	-0.309*	-0.218	+0.174
No. of Tillers	-0.224	+0.126	-0.117	+0.005
No. of Spikelets	-0.201	-0.109	-0.030	+0.016
Spike Length	-0.262	-0.169	+0.081	-0.058
Seed-set	+0.278	+0.106	+0.100	-

n = 40; * indicates significant at 5% level

Table II.44. Simple Correlation Coefficients between Cytological Features and Morphological Characters in High and Low Populations.

Morphological Characters	Population	Cytological Features			Seed-set
		Chiasma Frequency	Disjunction Index	Regular Tetrads	
Plant Height	HIGH	+0.073	+0.007	+0.081	+0.229
	LOW	+0.027	-0.262	-0.266	+0.246
No. of Tillers	HIGH	-0.108	-0.259	-0.156	-0.066
	LOW	-0.376*	-0.373*	-0.121	+0.085
No. of Spikelets	HIGH	+0.160	+0.196	+0.198	+0.358*
	LOW	-0.416*	-0.472*	-0.633***	+0.152
Spike Length	HIGH	+0.128	+0.091	+0.217	+0.308*
	LOW	-0.233	-0.221	-0.422*	-0.263
Seed-set	HIGH	+0.436**	+0.420**	+0.471***	-
	LOW	-0.264	-0.411*	-0.245	-

n = 40 for the high population

n = 23 for the low population

for more vigorous plants. We would, therefore, expect that with the improvement in meiotic regularity, there is also an improvement in plant vigour. This will break the supplementary relationship between meiotic regularity and plant vigour found in the unselected population. As a result no significant canonical correlation between the two sets of characters will be expected. This is what we find with the "high" population where the canonical correlation is the lowest among the three populations (P = 20%-30%). For the same reasons, all the simple correlation coefficients, which were negative in the unselected population (table II.43), are positive in the "high" population (table II.44), yet the seed-set shows positive correlations with each of the cytological and the morphological characters as in the unselected population.

The low population, being selected for irregular meiosis, also shows a reduction in the canonical correlation (P = 10%-20%, table II.42a) but the simple correlations between cytological features and morphological characters shown in table II.44 are all negative with one exception (encircled). It may be borne in mind that the chromosome complement in some members of the "low" population was abnormal. As a result seed-set is negatively correlated with each of the three cytological features (bottom line in table II.44). On the other hand, seed-set is positively correlated with the morphological characters in the "low" population with the exception of spike length.

DISCUSSION

Two groups of factors that affect chromosome association can be recognised. One is the homology, the chemical and structural similarity between the chromosomes themselves and the other is the cellular environment during meiosis (Stebbins, 1970). The evidence that cellular environment affects pairing is recognised either by a drastic change in the environmental agents, for example, temperature, nutrients, etc. (see section three) or by genes that apparently control enzymatic reactions required for regular chromosome pairing, for instance the 5B chromosome in wheat (Riley, 1960). In rye there is no evidence for such specific gene or genes, as in 5B chromosome of wheat, that control chromosome association throughout the entire genome. This leaves us with two other avenues of explanation, namely, the chromosome homology and genic factors that influence pairing indiscriminately. The effect of the latter is manifested by lower chiasma frequency due to individual recessive genes in homozygous conditions causing failure of initial chromosome association at zygotene-pachytene or the failure of chiasma formation even if there is synapsis at zygotene-pachytene (for example see Mehra & Rai, 1972). Since the average frequencies of chiasmata and univalents did not differ significantly between populations (table II.10a), the evidence for recessive genes affecting pairing indiscriminately is lacking in the present materials. Therefore, the differences in pairing pattern, between the

populations investigated, seems to be primarily due to chromosome homology.

The role of chromosome homology in the degree of pairing is well known. In many interspecific hybrids between parents having chromosomes sufficiently alike so that they can pair, but different enough to reduce the chiasma frequency in the undoubled hybrid, the amount of pairing is reduced. When such hybrids are doubled, pairing is largely confined to chromosomes derived from the same parent (for example, see Upcott, 1939 on Primula kewensis). Such preferential pairing may form exclusively bivalents by complete elimination of homeologous pairing or they may form varying numbers of multivalent configurations involving fully homologous and homeologous chromosomes. In polyploid forms of rye Timmis & Rees (1971) observed a preference for chromosome association in "pairs". From this the above authors suggested that in autotetraploid rye an increase in bivalents at the expense of multivalents would be effective and more readily achieved, by reinforcing the formation of exclusive "pairs" at pachytene. Sybenga (1972) reports, "gross structurally identical but genetically slightly different chromosomes of the same species, although they pair very efficiently in diploid, show considerable variation with respect to association in a competitive situation in trisomics". He further states that the two arms of the same chromosome are independent in respect to their pairing properties. By the same token it is reasonable to suppose

that chiasma distribution in each of the seven sets of rye chromosomes is independent of other sets.

The results obtained for the three rye populations can be explained both in terms of homology and independent chiasma properties for each set of rye chromosomes.

It has been concluded earlier that "restricted" chiasma distribution between two of the four homologous chromosomes leads to increased bivalent frequency in the pollen mother cells whereas with "free" distribution of chiasmata among the four homologous chromosomes, quadrivalent frequency is increased. The former is the case where two of the four homologous chromosomes have differentiated, structurally or chemically, in an identical way from the other two homologues which might have remained in the original form. In such a case the pairs of differentiated and normal chromosomes will form two separate bivalents with adequate number of chiasmata being available. This is a situation similar to preferential pairing in inter-specific hybrids. If on the other hand, all the four homologous chromosomes are identical, as would be expected in a true auto-tetraploid, increased chiasma frequency will favour quadrivalent formation. It is on these bases, the "high" population showed significant correlation between quadrivalent frequency and chiasma frequency while in the "low" and the unselected populations bivalent frequency showed significant correlation with chiasma frequency. According to these correlations and with average

chiasma frequency being similar in the three populations, one would expect a higher average for quadrivalents and lower average for bivalents in the high population. But in actual case this was just opposite, i.e. the mean quadrivalent frequency was significantly less and the mean bivalent frequency was greater in the "high" population as compared to those in the unselected and the "low" populations.

It is not difficult to see why it can be so. We may recall the earlier assumption that the chiasma distribution pattern in each of the seven sets of rye chromosomes is independent of the other sets. Now if at the start of the selection programme, of the two diploid gametes fused together, one parental gamete carried two chromosomes which were differentiated, structurally or chemically, from the two chromosomes contributed by the other parental gamete, we would expect some preferential pairing so that the two chromosomes derived from the same parent form a bivalent in most cases. Henceforth we followed sib-crossing. This means that following every sib-crossing, two gametes each carrying one chromosome from each original parent fused together and thus maintained the bivalent formation. In other words, bivalent frequency will remain roughly constant for this particular set of chromosomes as sib-crossing will be continued.

In other sets of chromosomes the four representatives may be strictly homologous where quadrivalent formation will be

favoured with increasing chiasma frequency. Conversely, these multivalents, being more sensitive to any change in the cellular environment (Section Three), a reduction in chiasmata will affect the quadrivalents to a greater extent than the bivalents. In other words, bivalent frequency remaining unchanged, the significant correlation between quadrivalents and chiasmata in the "high" population is mainly due to those chromosome sets where the four homologues are identical. Therefore, the significantly lower average of quadrivalents does not contradict the stronger correlation between chiasma frequency and quadrivalent frequency.

Another significance of the correlation between quadrivalent frequency and chiasma frequency in the "high" population is that we would not expect any further increase in bivalent formation in this population. Because the above correlation is a feature of a true autotetraploid as found in inbred lines (Hazarika & Rees, 1967).

On the other hand, there exists a great deal of chromosomal "differentiation" in the unselected and the "low" populations, which with increasing chiasmata would provide the scope for increasing the frequency of bivalents. This is substantiated by the significant correlation between chiasma frequency and bivalent frequency. The increase in bivalent frequency may be achieved to the same extent as, or to a greater or lesser extent than

the "high" population depending on the number of chromosome sets in which "differentiation" is present. For example, if each of the seven sets of chromosomes has differentiated in pairs, we can expect 14 bivalents. Any deviation from this will decrease the average bivalent frequency.

As regards seed-set, its dependence on meiotic behaviour and plant vigour varied for the three populations. The results suggest that both cytological and physiological factors influence seed-set in autotetraploid populations and the two sets of factors are at a "balance" in the advanced unselected population and supplement each other in determining seed-set. This balance can be easily upset by selection pressure without any serious effects on seed-set. This indicates, on one hand, independent genetic control of cytological and physiological factors and on the other, the need for selection of both sets of factors for the improvement in fertility in a population.

S E C T I O N T H R E E

EFFECTS OF EXTERNAL ENVIRONMENTAL FACTORS ON MEIOTIC CHROMOSOME ASSOCIATION IN AUTOTETRAPLOID RYE

INTRODUCTION

Variations in the nuclear phenotype due to changes in environmental factors are well known. In a recent paper Flannagan & Jones (1973) have demonstrated that the various experimental agents which reduce the growth and development of the plant, also reduce chromosome volume and total nuclear protein. Changes in the nuclear phenotype may occur independently of any variation in nuclear DNA amount (Bennet & Rees, 1969) or may sometimes include changes in DNA content (Evans, 1968; Durrant, 1971; Durrant & Timmis, 1973). In addition some of the induced phenotypic changes in the plant have been reported to be permanent and heritable (see Brink, 1958; Durrant, 1962a; Hill, 1965; Perkins et al, 1971; Durrant & Timmis, 1973).

The effects of external environmental factors on meiotic properties and recombination have been known in both plants and animals. There is a considerable number of reports on the effects of temperature changes on meiotic behaviour. These include chiasma frequency and distribution (Dorwick, 1957; Wilson, 1959a & b; Elliot, 1955 & 1958), chromosome coilings and nuclear inactivation (Jain, 1957) and meiotic duration

and pollen maturation (Darlington, 1940; Dorwick, 1957; Wilson, 1959b).

Other investigations have demonstrated the effects of ionic environment and mineral treatments on chromosome pairing behaviour at meiosis. Thus Levine (1955) and Eversole & Tatum (1956) observed that ionic changes in the nutrients supplied can have predictable consequences at meiosis. Steffensen (1957) reported that in Tradescantia cation imbalance caused by sub-optimal calcium supply increased chromosomal aberrations and suggested that calcium plays an important part in chromosome stability. Law (1963) demonstrated the effects of potassium in increasing the chiasma frequency and chiasma stability in Lolium temulentum. Similar results were obtained by Bennet & Rees (1970) in rye and Fedak (1973) in barley in response to phosphate treatments.

One would note that all the above mentioned investigations were carried out exclusively with diploid materials. Significantly very little information is available as to the effects of environmental factors on autopolyploids. Myers (1943) in his study on Dactylis noted that some factor or factors of environment had measurable effects on quadrivalent frequency and the effects of those factors were not the same for all genotypes. He further stated that soil heterogeneity had no effect on quadrivalent frequency or on chiasma frequency. In autotetraploid rye Pao & Li (1948) observed that high temperature

treatment (36°C) for 24 hours markedly increased the frequency of univalents with a decrease in quadrivalent frequency. On the other hand, Sparnaaij, et al, (1968) and Kho & Baër (1973) reported that changes in temperature treatment in tetraploid freesia during flowering and pollination affected the fertility of individual flowers and the ultimate seed-yield of the plant. Meiotic studies were not apparently undertaken in these materials. In tetraploid rye Ellerström & Sjodin (1963) reported an increase in seed-set following an application of calcium nitrate to the crop during the flowering time. In their opinion, the increased seed-set was due to the favourable conditions for seed-development provided for by the application of the fertiliser.

However, studies on the effects of external environmental factors in autopolyploids may be of interest for two reasons. Firstly, such investigations may provide a better understanding of meiotic stability of tetraploid genotypes. Secondly, changes in environmental factors, especially nutrient treatments, have been shown to increase chiasma frequency (Steffensen, 1957; Law, 1963; Bennet & Rees, 1970; Fedak, 1973) which, in turn, is an important component of meiotic stability (Rees & Thompson, 1956; Bennet & Rees, 1970). It, therefore, seems that it may well be possible to improve the chromosome pairing behaviour in autotetraploids, at least temporarily, by the influence of external environmental agents. On the other hand, based on the evidence that heritable changes can sometimes be

induced by external agents (Durrant, 1962a, Hill, 1965; Perkins, et al, 1971), it is perhaps too early to discount the possibilities of manipulating the chromosome pairing system in tetraploids for better meiotic stability by the influence of external factors.

In the present study two separate experiments were conducted to observe the effects of temperature and nitrogen on meiotic chromosome behaviour of tetraploid rye. Each genotype was cloned and subjected to different treatments so that the differences observed between treatments were mainly due to environmental effects.

MATERIALS

Seeds of tetraploid spring rye, collected from open pollinated plants, were sown in 5" pots. A single seed was sown in each pot containing John Innes Compost No. 2. Since it was intented to establish clonal material of each genotype, the seedlings were raised in a glasshouse during the winter months (from December onwards) in order to provide a longer period of vegetative growth and tillering. When the seedlings produced several tillers (5 to 10), the tillers were divided into three units in order to provide three replicates of each genotype. During splitting of tillers, care was taken so that each unit had sufficient root system to establish itself when transplanted into individual 5" pots. After transplanting, a layer of peat was spread over the soil surface of the pot. The plants were watered regularly and left to grow in the greenhouse. Clones of a large number of plants were raised in this way for use in the temperature and the nitrogen experiments.

TEMPERATURE EXPERIMENT

Methods

When the emergence of the first flag leaf was noticed, the three replicates of each genotype were treated with three different conditions of temperature as outlined in the table below:

TREATMENT	PLACE	TEMPERATURE	DAY LENGTH
I	Outdoors	$12^{\circ}C$ - $18^{\circ}C$ (approx.)	May/June
II	Growth room	$18^{\circ}C$ - Day $16^{\circ}C$ - Night	16 Hours
III	Growth room	26° Constant	16 Hours

Young spikes were fixed in acetic alcohol (1:3) from each treatment. Later the fixations were transferred to 70% ethyl alcohol and stored in a refrigerator ($3^{\circ}C$ to $5^{\circ}C$) until they were cytologically examined. The technique of slide preparation has been described earlier (Section One). The following observations were made from the spikes of eight clones under each treatment:

(1) Number of Chiasmata in 20 PMCs

(2) Number of Quadrivalents in 20 PMCs

(3) Number of Trivalents in 20 PMCs

(4) Number of Bivalents in 20 PMCs

(5) Number of Univalents in 20 PMCs

(6) Percentage of Regular Metaphase-I Cells Scored from Three Anthers of One Floret (about 200 MI Cells per anther)

(7) Percentage of Regular Tetrads from Six Anthers of Two Florets (about 200 tetrads per anther)

RESULTS

Chiasma Frequency

Table III.1a shows the effects of temperature treatment on chiasma frequency in different clones of tetraploid rye while table III.1b gives the analysis of variance. The variance analysis shows highly significant differences between treatments. "L.S.R." test for treatments revealed that the difference between treatment I (outdoor temperature) and treatment II ($16^{\circ}C$ - $18^{\circ}C$) is not significant, although the mean value for the latter treatment is slightly higher. This indicates that $16^{\circ}C$ - $18^{\circ}C$ is very close to the optimal temperature requirement for the rye material. Treatment III ($26^{\circ}C$), however, shows a significant reduction in chiasma frequency over treatments I and II. This is in good agreement with the results obtained by other workers in that high temperature reduces chiasma frequency.

It is of interest to note that all the clones are not affected uniformly by the temperature treatments. This can be judged from the highly significant interaction item ($P < 0.001$) in the analysis of variance table. L.S.R. test for interaction revealed that the effects of treatments I and II are similar in all the clones while treatment III shows a significant reduction in chiasma frequency in all but clone Nos. 4 and 7. The constancy of these two clones in chiasma frequency over the range of temperature may suggest that they have a greater

Table III.1a. Effects of Temperature on Chiasma Frequency per PMC in Clones of Tetraploid Rye.

CLONE No.	TREATMENT I (Outdoor Temp.)	TREATMENT II ($16^{\circ}C$-$18^{\circ}C$)	TREATMENT III ($26^{\circ}C$)	MEAN
1	24.40	25.05	22.65	24.033
2	26.30	26.25	23.60	25.383
3	25.05	26.10	23.25	25.133
4	25.60	25.80	24.95	25.450
5	25.45	25.55	25.50	25.500
6	24.75	24.45	22.30	23.833
7	24.25	24.45	23.65	24.083
8	25.75	24.65	23.60	24.667
Mean	25.195	25.275	23.813	

Table III.1b. Analysis of Variance of Chiasma Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	108.0896	41.480	<0.001
Clones	7	29.1854	11.200	<0.001
Interaction (Treatment x Clone)	14	7.6229	2.925	<0.001
Error	456	2.6058		

genotypic stability with respect to temperature effects. This is what one would expect from genotypes collected from an outbred heterogeneous population.

Quadrivalent Frequency

The frequencies of quadrivalents under different treatments are shown in table III.2a and table III2b. gives the analysis of variance. There are significant differences in the frequencies of quadrivalents between treatments ($P < 0.05$). Unlike chiasma frequency, the mean frequencies of quadrivalents under treatment II ($16^{\circ}C$ - $18^{\circ}C$) is significantly reduced from that in treatment I (Outdoor temperature). The reduction is more pronounced in treatment III ($26^{\circ}C$) and corresponds with the reduction in chiasma frequency (table III.1). It is, however, noted that the difference in quadrivalent frequency between treatment II and III is insignificant in contrast to significant differences in chiasma frequency observed earlier. These results suggest that in the materials used in the experiment, there is no consistent relationship between chiasma frequency and quadrivalent frequency. The interaction item is insignificant which indicates that the responses of different clones to temperature changes with regard to quadrivalent frequency are uniform for all genotypes.

Table III.2a. Effects of Temperature on Quadrivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	2.75	2.10	1.45	2.100
2	3.55	2.25	2.50	2.767
3	2.35	1.90	2.00	2.083
4	2.60	2.75	2.80	2.717
5	2.50	2.50	2.15	2.383
6	2.15	2.35	2.15	2.217
7	1.95	2.05	1.85	1.95
8	2.55	1.90	2.05	2.167
Mean	2.550	2.225	2.119	

Table III.2b. Analysis of Variance of Quadrivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	8.0771	6.432	<0.05
Clones	7	5.4211	4.317	<0.001
Interaction (Treatment x Clone)	14	2.1176	1.686	not significant
Error	456	1.2558		

Trivalent Frequency

The changes in the frequency of trivalents in the clones due to different temperature are shown in table III.3a. The analysis of variance appears in table III.3b. It is observed that treatment III ($26^{o}C$) shows a significant increase in trivalent frequency over treatments I and II ($P < 0.05$). This increase in trivalent frequency with a parallel decrease in quadrivalent frequency, as already shown, suggests that the increase in trivalents is due to the failure of quadrivalent formations. From this one can conclude that high temperature inhibits quadrivalent formation.

It is, however, noticeable that the difference between the means for treatment I and II is insignificant, suggesting once more that the artificial temperature provided by treatment II is optimal.

Bivalent Frequency

From table III.4a & b it is clear that within the range of temperature used, there is no significant change in bivalent frequency. It should, however, be pointed out that an accurate estimate of the changes in bivalent frequency is extremely difficult for two reasons. Firstly, temperature changes may inhibit multivalent formations and may form bivalents instead. Secondly, there may be some failure in bivalent formation giving rise to two univalents. The effects of these two possibilities

Table III.3a. Effects of Temperature on Trivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	0.25	0.30	0.50	0.350
2	0.15	0.10	0.45	0.233
3	0.20	0.15	0.35	0.233
4	0.15	0.30	0.30	0.250
5	0.10	0.10	0.15	0.117
6	0.25	0.35	0.60	0.400
7	0.30	0.25	0.35	0.300
8	0.10	0.20	0.35	0.217
Mean	0.188	0.219	0.381	

Table III.3b. Analysis of Variance of Trivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatment	2	1.7313	6.876	<0.01
Clones	7	0.4560	1.811	not significant
Interaction (Treatment x Clone)	14	0.1051	0.417	not significant
Error	456	0.2518		

Table III.4a. Effects of Temperature on Bivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	7.90	9.00	9.60	8.833
2	6.55	9.25	7.95	7.917
3	8.85	9.90	9.15	9.30
4	8.40	7.90	7.80	8.033
5	8.70	8.75	9.35	8.933
6	8.95	8.40	8.00	8.450
7	9.45	9.20	9.50	9.383
8	8.65	9.75	8.95	9.117
Mean	8.431	9.019	8.787	

Table III.4b. Analysis of Variance of Bivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	14.0146	2.905	Not significant
Clones	7	18.6559	3.868	<0.01
Interaction (Treatment x Clone)	14	8.4836	1.759	<0.05
Error	456	4.8237		

are confounded in the overall bivalent frequency observed. However, from the significant decrease in quadrivalents with a corresponding increase in trivalents, as we have already seen, it can be assumed that the increase in bivalent frequency as a result of inhibition of multivalent formations is insignificant. Based on this assumption it would appear that temperature changes did not affect bivalent frequency (table III.4b). From this it would appear that bivalents are relatively stable type of configurations compared to quadrivalents. This will be dealt with under discussion.

In perspective to the significant decrease in chiasma frequency in treatment III (Table III.1), bivalent frequency also could have been reduced, but this was not so. This can be explained as follows. If as a result of chiasma reduction, there are more rod bivalents with one chiasma, instead of rings with two chiasmata, the total number of bivalents may still remain unchanged. A test in this regard was made and the frequency of ring bivalents was reduced from 7.60 per PMC in treatment II to 6.48 per PMC in treatment III and the difference was highly significant ($P < 0.001$).

The interaction item in the analysis of variance was significant ($P < 0.05$, table III.4b). The L.S.R. test for interaction revealed that in each clone the differences in bivalent frequency between treatments were insignificant except in clone nos. 1 and 2. Deviations of these two clones

from the general pattern exhibited by all other clones, may indicate the genetic instability of these two clones with respect to pairing behaviour or the changes may be due to sampling error. If it was due to the latter cause, the error could be reduced by using replicated samples from each individual plant.

Univalent Frequency

Univalents, being the ultimate results of the changes in all other configurations and also being freed from confounding effects, as with bivalents, give a better picture of the treatment effect. The frequencies of univalents under different treatments have been shown in table III.5a and b. It is clear from the tables that the differences between treatments is highly significant ($P < 0.001$). Similarly, the interaction item is also highly significant ($P < 0.001$). From a closer examination of the data by means of L.S.R. test, it was observed that the difference between treatments I and II is non-significant for each clone with the exception of clone Nos. 4, 5 and 7. These three clones, therefore, show stability in univalent frequency at differing temperature. Clone Nos. 4 and 7 were also observed to be stable with regard to quadrivalent frequency (table III.2).

Regular Metaphase-I Cells

The percentage figures for regular metaphase-I cells,

Table III.5a. Effects of Temperature on Univalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	0.45	0.70	1.50	0.883
2	0.25	0.20	0.75	0.400
3	0.30	0.15	0.65	0.367
4	0.35	0.30	0.30	0.318
5	0.30	0.20	0.25	0.250
6	0.55	0.75	1.60	0.933
7	0.40	0.65	0.55	0.533
8	0.20	0.30	0.85	0.450
Mean	0.375	0.406	0.806	

Table III.5b. Analysis of Variance for Univalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	9.9021	68.623	<0.001
Clones	7	4.1893	29.032	<0.001
Interaction (Treatment x Clone)	14	1.1902	8.248	<0.001
Error	456	0.1443		

Table III.6a. Effects of Temperature on Regular Metaphase-I Cells (Angular Values) in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	62.77	57.06	50.86	56.877
2	70.34	77.82	64.99	71.051
3	70.33	73.99	63.22	69.179
4	71.21	72.32	63.85	69.127
5	71.89	70.81	67.08	69.926
6	57.31	61.03	46.55	54.964
7	64.04	63.11	61.34	62.830
8	74.20	67.75	58.34	66.764
Mean	67.755	67.894	59.530	

Table III.6b. Analysis of Variance of Regular Metaphase-I Cells.

ITEMS	D.F.	M.S.	F	P
Treatments	2	556.7598	160.243	<0.001
Clones	7	346.9282	99.851	<0.001
Interaction (Treatment x Clone)	14	30.2678	8.712	<0.001
Error	48	3.4745		

scored from three anthers of a floret, were transformed to angular values for use in the variance analysis. Thus table III.6a gives the angular values under each treatment for the respective clones and the analysis of variance is shown in table III.6b. There are significant differences between the treatment means ($P < 0.001$) but again the difference between treatment I and II is insignificant. The interaction item is also highly significant suggesting that the effect of temperature was not uniform for all genotypes. In particular clone No. 7 has remained stable with regard to the proportion of regular metaphase-I cells over the range of temperature treatment.

Regular Tetrads

The percentage figures for regular tetrads expressed in angular values are shown in table III.7a for individual clones under different temperature treatment. The analysis of variance in table III.7b shows that the treatment means differ significantly ($P < 0.001$). Unlike earlier observations, the difference between treatment I and II is significant here, the difference, however, being much smaller compared to the figure obtained for treatment III. L.S.R. test showed that in most of the clones the difference between treatment I and II is insignificant. The lower mean value for treatment II compared to that of treatment I is mainly due to the drastic reduction in the proportion of regular tetrads in clone No. 1

Table III.7a. Effects of Temperature on Regular Tetrads (Angular Values) in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I	TREATMENT II	TREATMENT III	MEAN
1	66.34	53.49	56.29	58.807
2	75.32	76.44	66.80	72.854
3	72.33	71.18	64.21	69.238
4	78.41	75.40	64.30	71.034
5	69.44	66.29	62.52	66.079
6	63.77	64.21	45.45	57.809
7	64.22	66.86	64.49	65.189
8	72.57	67.54	59.72	66.609
MEAN	69.710	67.675	60.473	

Table III.7b. Analysis of Variance of Regular Tetrads.

ITEMS	D.F.	M.S.	F	P
Treatments	2	1130.6250	175.912	< 0.001
Clones	7	521.0813	81.074	< 0.001
Interaction (Treatment x Clone)	14	96.2263	14.972	< 0.01
Error	120	6.4272		

under treatment II, which is even lower than the figure obtained for treatment III. This kind of rather unusual change points to the variation between spikes and emphasises the need for replicated samples.

It is, however, interesting to note again that clone No. 7 does not show any significant variation due to treatment effect. This clone has shown persistent stability in all other meiotic characters and would, therefore, appear to be the most stable genotype.

NITROGEN EXPERIMENT

Methods

The clonal material was established in the same way as in the case of temperature experiment. But after dividing the tillers into three units, one unit was grown in 5" pot containing John Innes Compost No. 2 and watered with tap water. This was regarded as treatment I, the control treatment. The other two units were grown in 5" pots containing vermiculite and washed sand. Since it was found difficult to keep the tillers erect and firmly anchored in the light vermiculite medium alone, a layer of washed sand was placed in between the two vermiculite layers, one at the bottom and the other at the top of the pot. One of these two pots was watered with Hoagland's Nutrient Solution containing all the nutrients and regarded as treatment II. The other pot was watered with Hoagland's Solution without nitrogen and regarded as treatment III.

Young spikes were fixed in acetic alcohol (1:3) from the three treatments and later examined cytologically in the same way as previously described.

RESULTS

In the present investigation the samples for meiotic studies were collected within 2 to 3 weeks of the commencement of nitrogen treatment. The plants under deficient nitrogen treatment (treatment III) became light green to pale yellow by the time the samples were collected and the colour was easily distinguishable from that of the dark-green plants under treatment I and II. Afterwards there was little growth or tillering in plants under the deficient treatment. As a result the first emerged spikes only were available for fixation. The effects of nitrogen deficiency in these spikes were not apparently serious because of the short period of exposure of the plants to the deficient treatment. It was, however, obvious that the spikes collected from these plants undoubtedly experienced a stress due to low level of nitrogen. Meiosis data obtained from these plants were, therefore, considered in terms of low nitrogen level rather than a deficient treatment.

Chiasma Frequency

Table III.8a shows the effect of nitrogen treatment on chiasma frequency on different clones and table III.8b gives the analysis of variance. Although there is a reduction in chiasma frequency in treatment III (low nitrogen), the difference with the control is just below the 5% level.

Table III.8a. Effects of Nitrogen Treatment on Chiasma Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low Nitrogen)	MEAN
1	25.60	25.80	25.15	25.517
2	25.75	26.25	26.15	26.050
3	25.60	25.25	26.05	25.633
4	25.90	25.95	25.85	25.900
5	26.15	25.55	26.00	25.900
6	26.15	25.25	25.05	25.483
7	25.30	25.40	24.65	25.117
8	26.10	25.35	24.75	25.400
Mean	25.819	25.600	25.456	

Table III.8b. Analysis of Variance of Chiasma Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	5.3315	2.808	not significant
Clones	7	5.7667	3.037	<0.01
Interaction Treatment x Clone	14	3.2622	1.718	<0.05
Error	456	1.8987		

The treatment x clone interaction is, however, significant ($P<0.01$). It will be seen from table III.8a that chiasma frequency has been reduced under low nitrogen treatment in all clones with the exception of clone Nos. 2, 3 & 5. However, from these results it appears that low nitrogen treatment has not drastically affected chiasma formation although a tendency towards reduced chiasma is apparent.

Quadrivalent Frequency.

Tables III.9a and b give the frequencies of quadrivalents under different treatment in the clones and their analysis of variance respectively. It is clear from the tables that as a result of low nitrogen treatment there is a highly significant reduction of quadrivalent frequency compared to the frequencies in the control as well as the artificially supplied nutrient treatment ($P<0.001$). The difference between the latter two is, however, insignificant. In contrast to insignificant changes in chiasma frequency, seen above, the highly significant changes in quadrivalents suggest, once again, that quadrivalent formation is not closely related to chiasma frequency in the materials. The results also show that quadrivalents are extremely vulnerable to the changes in nitrogen level.

Bivalent Frequency

Table III.10a gives the bivalent frequencies of individual

Table III.9a. Effects of Nitrogen Treatments on Quadrivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Now nitrogen)	MEAN
1	2.95	2.55	2.75	2.750
2	2.60	3.40	2.35	2.783
3	2.05	2.80	2.60	2.483
4	2.80	3.40	2.35	2.850
5	3.25	3.05	2.15	2.817
6	2.90	2.70	2.10	2.567
7	2.25	2.50	2.85	2.200
8	2.85	2.70	2.40	2.650
	2.706	2.887	2.319	

Table III.9b. Analysis of Variance of Quadrivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	13.5063	10.077	<0.001
Clones	7	2.8417	2.120	<0.05
Interaction (Treatment x Clone)	14	2.2015	1.643	not significant
Error	456	1.3404		

Table III.10a. Effects of Nitrogen Treatments on Bivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low nitrogen)	MEAN
1	7.95	8.70	8.20	8.283
2	8.60	6.90	9.05	8.183
3	9.50	8.05	8.50	8.683
4	8.10	7.00	8.90	8.000
5	7.15	7.45	9.35	7.983
6	7.85	8.35	9.20	8.467
7	9.15	8.65	9.90	9.233
8	8.00	8.30	8.75	8.350
Mean	8.287	7.952	8.981	

Table III.10b. Analysis of Variance of Bivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	46.0896	9.053	<0.001
Clones	7	10.0783	1.980	not significant
Interaction (Treatment x Clone)	14	8.6158	1.692	not significant
Error	456	5.0913		

treatments and clones and table III.10b gives their analysis of variance. It is clear that there is a highly significant increase in bivalent frequency in the low nitrogen treatment over the control and the artificially supplied treatments ($P < 0.001$). The difference between the latter two is again insignificant. A large reduction in quadrivalent frequency, as observed earlier in table III.9, with a concurrent increase in bivalent frequency suggests that with low nitrogen treatment chiasmata have been redistributed in favour of bivalent formation. Changes in the frequencies of different configurations as a result of chiasma redistribution, rather than changes in chiasma frequency has already been reported by Crowley & Rees (1968) in auto-tetraploid Lolium perenne. The above results, therefore, suggest that the low nitrogen treatment has induced bivalent formation at the expense of quadrivalents and these changes are not due to changes in chiasma frequency. This can be further confirmed if there is no significant increase in trivalent frequency with a reduction in the frequency of quadrivalents.

Trivalent Frequency.

The frequencies of trivalents under different treatments and clones have been set out in table III.11a while table III.11b gives the variance analysis. From the tables it is clear that there is no significant difference between treatment means. This is what we would expect from the absence of any significant

Table III.11a. Effects of Nitrogen Treatment on Trivalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low nitrogen)	MEAN
1	0.05	0.05	0.10	0.067
2	0.10	0.10	0.10	0.100
3	0.20	0.15	0.15	0.167
4	0.15	0.10	0.20	0.150
5	0.15	0.20	0.15	0.167
6	0.15	0.10	0.25	0.167
7	0.15	0.15	0.15	0.150
8	0.15	0.10	0.20	0.150
Mean	0.138	0.119	0.163	

Table III.11b. Analysis of Variance of Trivalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	0.0770	0.581	not significant
Clones	7	0.0807	0.608	not significant
Interaction Treatment x Clone)	14	0.0271	0.204	not significant
Error	456	0.1328		

change in chiasma frequency but with a significant change in quadrivalent frequency. Because an increase in trivalent frequency would be accompanied by a reduction in chiasma frequency as was observed with the temperature experiment.

Univalent Frequency

The univalent frequencies with respect to different clones and treatments are given in table III.12a. The analysis of variance in table III.12b shows that none of the items is significant. This suggests that nitrogen treatment did not have any detectable effect on univalent formation.

Regular Metaphase-I Cells

In table III.13a the angular values for regular metaphase-I cells of individual treatments and clones are presented. The variance analysis based on the angular values shows no significant difference between the control and the artificially supplied nutrient treatment. The mean value for the low nitrogen treatment is, however, significantly different from that of the two other treatments ($P < 0.001$). With regard to insignificant differences for chiasma frequency (table III.8) and univalent frequency (table III,11), the results obtained for regular M-I cells seem incompatible. With univalent frequency, however, the situation may be considered somewhat different. Because the regular M-I cells

Table III.12a. Effects of Nitrogen Treatment on Univalent Frequency in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low Nitrogen)	MEAN
1	0.15	0.25	0.30	0.233
2	0.10	0.30	0.20	0.200
3	0.20	0.25	0.15	0.200
4	0.15	0.10	0.20	0.150
5	0.25	0.30	0.25	0.267
6	0.25	0.20	0.45	0.233
7	0.25	0.15	0.35	0.250
8	0.15	0.30	0.20	0.217
Mean	0.188	0.231	0.263	

Table III.12b. Analysis of Variance of Univalent Frequency.

ITEMS	D.F.	M.S.	F	P
Treatments	2	0.3146	0.9868	not significant
Clones	7	0.1497	0.4696	not significant
Interaction (Treatment x Clone)	14	0.0979	0.3071	not significant
Error	456	0.3188		

were measured in terms of percentages of PMCs without univalent(s) whereas the univalent frequency is the number of univalents per PMC. If there are fewer PMCs with several univalents and vice versa the observed discrepancy between the two properties could be accounted for. But such a condition would affect chiasma frequency, for every single univalent in a PMC there will be a loss of at least one chiasma. This warrants a closer examination of the situation. It will be seen from table III.13 that the difference between the control and the low nitrogen treatment is insignificant for every single clone with the exception for clone No. 2 (L.S.R. Test) while the difference between the artificially supplied nutrient and the low nitrogen treatment is significant for clone Nos. 4 and 6 only. This sort of spurious differences apparently reflect the variation caused by sampling error. It would be recalled that the percentages of regular M-I cells were based on only three anthers collected from a single floret. Data collected from such a restricted sample will very much depend on chance, especially if factors like prevailing temperature cannot be controlled during meiosis. During the present investigation the plants were grown inside the greenhouse where the temperature varied considerably on a warm sunny day than from a cool rainy day. If data were collected from several florets and different spikes of the same plant, the variation due to sampling error could be reduced to a large extent. That the discrepant variations in regular M-I cells were mainly due to sampling error can

Table III.13a. Effects of Nitrogen Treatments on Regular Metaphase-I Cells (Angular Values) in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low nitrogen)	MEAN
1	72.23	71.54	71.25	71.671
2	75.65	72.28	69.38	72.436
3	70.30	68.89	71.64	70.276
4	68.73	75.35	67.04	70.373
5	70.23	70.38	67.86	69.489
6	69.45	70.46	65.85	68.587
7	68.51	66.93	66.99	67.479
8	67.06	66.14	64.69	65.966
Mean	70.270	70.246	68.087	

Table III.13b. Analysis of Variance of Regular Metaphase-I Cells.

ITEMS	D.F.	M.S.	F	P
Treatment	2	37.7110	7.2730	<0.01
Clones	7	41.2681	7.9590	<0.001
Interaction (Treatment x Clone	14	12.3378	2.3795	<0.01
Error	48	5.1851		

be examined from the figures below, (table III.13) obtained for regular tetrads where at least six anthers from two different florets were used and the results obtained there, as will be shown, fairly agree with what we have observed before, except regular M-I cells.

Regular Tetrads

The percentages of regular tetrads expressed in angular values for different clones and treatments are given in table III.14a. Their variance analysis in table III.14b shows insignificant differences between treatment means. From this it can be concluded that the low nitrogen treatment did not have any serious effects on meiotic regularity, as we have observed with chiasma frequency, quadrivalent, trivalent, bivalent and univalent frequencies.

Theoretically one would expect a similarity between the frequencies of regular M-I cells and regular tetrads, because micronuclei in tetrads are the direct results of univalents at M-I. The disagreement between the two features seems to be mainly due to high degree of sampling error caused by restricted sample size in the case of regular M-I cells.

Table III.14a. Effects of Nitrogen Treatments on Regular Tetrads (Angular Values) in Clones of Tetraploid Rye.

CLONE NO.	TREATMENT I (Control)	TREATMENT II (Nutrients supplied artificially)	TREATMENT III (Low nitrogen)	MEAN
1	72.17	67.51	70.01	69.897
2	72.42	59.74	68.64	66.931
3	66.99	66.90	68.28	67.389
4	71.36	71.62	71.37	71.451
5	70.77	71.02	68.22	70.003
6	67.99	69.15	67.53	68.223
7	65.62	67.84	66.08	66.514
8	65.48	65.73	65.84	65.680
Mean	69.100	67.439	68.244	

Table III.14b. Analysis of Variance of Regular Tetrads.

ITEMS	D.F.	M.S.	F	P
Treatments	2	33.1188	0.8420	not significant
Clones	7	72.324	1.8388	not significant
Interaction (Clone x Treatment)	14	40.6729	1.0341	not significant
Error	143	39.3327		

DISCUSSION

Temperature Experiment

The adverse effects of high temperature treatment on chiasma frequency and meiotic pairing behaviour were confirmed in tetraploid rye. The results were in good agreement with the findings reported earlier for both diploid and tetraploid organisms, in particular Pao & Li (1948)'s observations that high temperature reduced the frequency of quadrivalents and increased univalents. The significant treatment x clone interaction in the variance analyses supports Myer's (1943) observation on Dactylis that the effects of environmental factors are not the same for all the genotypes. That is, the individuals within a natural population show significant variation in their responses to environmental factors.

Two interesting facts have emerged from the temperature experiment. One is the optimal temperature requirement for the rye material which was found to be $16^{o}C$ - $18^{o}C$ and the other is the ability of certain genotypes to withstand moderate range of temperature without any significant effect on meiotic behaviour. The latter findings may provide means of identifying meiotically stable genotypes from within a tetraploid population. Screening of such genotypes may eventually lead to the establishment of a stable tetraploid

population. It was, however, felt that an investigation in this respect should be conducted by using replicated samples from each individual plant.

It was shown that with increased temperature, chiasma frequency, the frequency of quadrivalents and the proportions of regular metaphase-I cells and regular tetrads were reduced. Parallel to this, as expected, trivalent and univalent frequencies were increased. On the other hand, bivalent frequency appeared to have remained unchanged. Bivalents are not only the most desired type of configurations for balanced gamete formation but also seem to be the most stable type of pairing configurations. This may be realised from the following facts.

Firstly, as a result of some reduction of chiasma frequency which may be caused by any of the environmental factors, there will be more rod bivalents formed instead of rings. It was found that under normal temperature condition (i.e. outdoor treatment), ring bivalents with two or more chiasmata comprise the bulk of the total number of bivalents (about 85% in the present material). If one accepts an average reduction of one chiasma per bivalent, rod-bivalents will be predominant. This would not change the total number of bivalents appreciably neither would this affect regular anaphase distribution.

Secondly, with a reduction in chiasma frequency quadrivalents

are at a much more serious disadvantage because of two reasons. (A) The number of chiasmata per quadrivalent in rye is low, usually 3 to 4 and the ratio of 3-chiasmate quadrivalents to 4 chiasmate quadrivalents (chain IV:ring IV) is about 2:3 as found in the present material under outdoor treatment. This would mean that a reduction of a single chiasma per quadrivalent would reduce at least 40% of the quadrivalents (i.e. 3-chiasmate chains) to trivalents plus univalents, while the remaining 60% being rings with 4 chiasmata would be expected to become chains. It is, therefore, apparent that a reduction of chiasmata would affect quadrivalents more than the bivalents. (B) The reduction of quadrivalent frequency may also stem from the pairing competition of the four homologous chromosomes at zygotene-pachytene. Since the formation of a quadrivalent involves competition among the homologues, it is conceivable that any interference by an external agent, like temperature, would only weaken the pairing efficiency of the four homologues. On the other hand, such pairing competition does not exist in the formation of a bivalent and consequently the two homologues will experience less difficulty in undergoing pairing with the interference by an external agent.

Nitrogen Experiment

Whether the artificially supplied nutrients may have an

effect on meiotic behaviour is not precisely known. Steffensen (1957) reported increased chromosomal abnormalities owing to ionic imbalance. Stubbe & Döring (1938) found that a fluctuation in the balance of nutrients has a bearing on chromosomal disturbances. In order to check such effects, a treatment with all the nutrients supplied artificially (Treatment II) was included in the experiment. The results suggest that there is no significant difference between the control (Treatment I) and the artificially supplied nutrient treatment (Treatment II) in any of the meiotic properties studied, therefore, the different treatments were directly comparable.

The various meiotic properties studied suggest that under the present experimental conditions low level of nitrogen did not increase meiotic disturbances to a significant extent but a tendency towards reduced chiasma frequency was observed. It, therefore, seems that if the plants were grown under nitrogen starvation for a relatively longer period, the irregularities in meiotic behaviour might become more pronounced. Walther (1959) observed a significant increase in meiotic disturbances in plants grown in nitrogen deficient media for a longer period of time, for about six weeks.

The results further showed that under low nitrogen treatment there was a significant increase in bivalent frequency, at the expense of quadrivalents. This change occurred without

any significant alteration in chiasma frequency. This was, therefore, interpreted as redistribution of chiasmata in favour of bivalent formations. Crowley & Rees (1968) reported an increase in quadrivalent frequency by a redistribution of chiasmata in autotetraploid Lolium.

The spurious variability in regular metaphase-I cells, in both nitrogen and temperature experiments, indicates the need for replicated sample from each plant. The variation in meiotic regularity between different flowers of the same spike may depend on developmental variation (Rees & Naylor, 1960), such as position of the flower in the spike as indicated by Walther (1959) or on the prevailing weather condition such as temperature during meiosis.

S E C T I O N F O U R

CHROMOSOMAL ABNORMALITIES

I. Aneuploidy

The occurrence of aneuploids in an autotetraploid population is due to the univalents and those multivalents which give rise to unequal chromosome separation at meiosis. A special point has been made for rye chromosomes that the chiasma frequency in quadrivalents is low (usually 3 to 4) and they are located terminally or sub-terminally and thus facilitates two-by-two chromosome separation at first anaphase (Røseweir & Rees, 1962; Hazarika & Rees, 1967). While this is true, it has to be pointed out that so far no tetraploid population has been reported, for rye in particular, where quadrivalent formation is essentially obligatory. This means, as normally found in any tetraploid rye material, that the pairing configurations in PMCs vary from univalents to quadrivalents, thus resulting in unequal chromosome disjunction at anaphase-I and subsequent production of aneuploids in the progeny. Therefore, until the chromosome association pattern can be modified either for obligatory quadrivalent formations or for obligatory bivalents with all the seven sets of rye chromosomes, the occurrence of aneuploidy in an autotetraploid population is inevitable.

There are several publications which report differences

in the frequencies of aneuploids in different populations as well as in different C-generations of the same original population. Muntzing (1954) observed 22.7% aneuploids in an early C-generation of the variety Dubbelstål and ten generations later Hagberg & Ellerström (1959) found a reduction to 14.7%. O'Mara (1943) reported 19% aneuploids in a freshly produced tetraploid population. In a later generation of the German variety Tetra Petkus Morrison (1956) found 13% aneuploids. In F_3 generations of two separate crosses between inbred lines Peggington (1971) found 6.9% and 10.8% aneuploids. The low frequency of aneuploids in these materials seems to be due to the selfing practised in the plants which had apparently affected the development of aneuploid zygotes to a greater extent than it had affected the development of zygotes with 28 chromosomes. This assumption is indirectly supported by the results of Ellerström & Sjodin (1963) who reported that an increase in seed-set, achieved by providing favourable conditions for zygotic development, is accompanied by increased aneuploid frequency. Moore's (1963) results also support this hypothesis.

In order to demonstrate the effect of meiotic regularity on aneuploid frequency in the progenies, Peggington (l.c.) selected plants for high and low disjunction index and his results indicate that the frequency of aneuploids can in fact be reduced by selection for meiotic behaviour.

Because of the ever increasing importance of polyploidy

in cultivars and because agronomically useful populations are likely to be mixtures of aneuploids and euploids (Shaver, 1962), it seems important to study the effects of aneuploidy not only on fertility but also the effect upon the frequencies of aneuploids in populations selected for meiosis behaviour as against the unselected populations.

In the present study three rye populations, subjected to varied selection influence (see Section II), were grown under the same environmental conditions and, therefore, any difference in the frequencies of aneuploids and the average performance of such plants in different populations should be attributable to the differences in meiotic behaviour and/or the type of selection applied to the respective population.

Results

The frequencies of euploids, aneuploids and structurally aberrant plants in the three populations were shown in table II.5 (Section Two). With the exclusion of the structurally aberrant plants and regarding the plants with chromosome fragments as one kind of aneuploids, the frequencies of euploids and aneuploids become slightly different and these are shown in table IV.1.

Table IV.1. Number and Frequency of Euploids and Aneuploids in High, Low and Unselected Populations.

POPULATIONS	CHROMOSOME NUMBER							TOTAL
	26	27	28	29	30	31	With fragments	
HIGH	-	16 6.78%	198 83.90%	18 7.63%	3 1.28%	-	1(27+1f) 0.43%	236
LOW	1 0.84%	8 6.73%	85 71.43%	22 18.49%	1 0.84%	1 0.84%	1(27+1f) 0.84%	119
UNSELECTED	-	5 4.51%	86 77.48%	18 16.22%	2 1.81%	-	-	111

From the above table, the frequencies of aneuploids in the "high", the "low" and the unselected populations are 16.10%, 28.57% and 22.52% respectively.

If one assumes that the frequencies of plants with different chromosome number in the absence of selection are represented by the figures in the unselected population (table IV.1), the corresponding figures in the "high" and the "low" populations would show the effects of selection upon aneuploid frequency. In other words, the frequencies in the unselected population provide the expected values which can be used to measure the effects of seleotion in the "high" and the "low" populations.

In order to do so, all the plants with known chromosome number in each population were grouped under four classes, namely, (i) 27-chromosome plants, (ii) 28-chromosome plants, (iii) 29-chromosome plants and (iv) other aneuploids (i.e. plants with 26, 30, 31 chromosomes and fragments). Chi-square test was done for each class of plants in the "high" and the "low" populations against the corresponding classes in the unselected population (table IV.2).

Table IV.2. Chi-Square Test for the Frequencies of Plants with Different Chromosome Number in the Selected High and Low Populations against the Corresponding Frequencies in the Unselected Populations.

Chromosome Number	HIGH		LOW	
	Chi-Square (1df)	P	Chi-Square (1df)	P
27	2.84	0.05-0.10	17.40	0.001
28	5.57	0.01-0.02	2.49	0.10-0.20
29	12.81	0.001	0.45	0.50-0.70
Other Aneuploids	0.02	0.80-0.90	0.36	0.50-0.70

From the above table it is evident that in the "high" population there is a significant increase of euploids ($P = 0.01-0.02$) with a highly significant decrease of 29-chromosome plants ($P < 0.001$).

The frequency of 27-chromosome plants was also decreased but the difference did not reach the significant level ($P = 0.05-0.10$) and virtually there was no difference for other aneuploids. In contrast, the low population shows a highly significant increase in the frequency of 27-chromosome plants ($P < 0.001$) but the difference for other chromosome classes are not significant, although an increasing trend for all aneuploid classes is apparent.

To relate these results with chromosome association pattern at meiosis, it would appear that the reduced level of aneuploids in the "high" population is due to the higher bivalent frequency of the euploid plants in the population (table II.10). It would further be noticed that in spite of/a similar quadrivalent frequency in the "low" and the unselected populations, the frequency of 27-chromosome plants in the "low" population is significantly higher. This may have resulted from the failure of some quadrivalents, possibly the straight rings, to give two-by-two disjunction with accompanying chromosome losses as laggards. However, what is apparent from these results is that bivalent formation better ensures the regular chromosome constitution in the gametes than quadrivalents. This, of course, contradicts the suggestion made by Roseweir & Rees (1962) and Nazarika & Rees (1967).

Performance of the Aneuploids in the Three Rye Populations.

The means and the standard errors of five morphological characters including seed-set of all the aneuploid plants in the three populations are presented in table IV.3a. In the same table the corresponding figures for an equal number of euploids selected at random are given. The comparisons between the euploids and the aneuploids of respective populations are given in table IV.3b.

Two characters, namely, plant height and seed-set of the aneuploid plants in each population show significant decreases compared to their euploids. But in the three morphological characters (no. of tillers, no. of spikelets and spike length), the difference between eu- and aneuploids varies for the populations. In particular, the aneuploids of the "high" population have similar tiller number, spikelet number and spike length to the euploids. In the "low" and the unselected populations, however, the differences between euploids and aneuploids are significant with one exception (tiller number) in the "low" population. The tables (IV.3a & b) also show that the differences between eu- and aneuploids of the unselected population are relatively higher than in the low population.

From table IV.4 it will be seen that the aneuploids from

Table IV.3a. Means ± Standard Errors for Morphological Characters of Euploids and Aneuploids under High, Low and Unselected Populations.

CHARACTERS	HIGH		LOW		UNSELECTED	
	Euploids (n=37)	Aneuploids (n=37)	Euploids (n=34)	Aneuploids (n=34)	Euploids (n=24)	Aneuploids (n=24)
Plant Height (cms)	127.00±2.635	115.00±3.289	136.09±2.449	110.00±3.239	143.88±2.015	113.79±3.648
No. of Tillers per plant	5.70±0.328	5.92±0.429	7.00±0.632	5.62±0.418	7.25±0.641	5.17±0.688
No. of Spikelets per spike	26.24±0.716	24.92±0.674	26.68±0.619	23.97±0.757	27.88±0.668	23.75±0.873
Spike Length (cms)	10.62±0.270	10.33±0.253	11.48±0.206	10.52±0.392	11.95±0.266	10.03±0.333
Seed-Set (Angular Values)	56.64±1.611	46.92±2.184	54.46±1.839	43.74±1.747	58.30±1.189	46.81±2.711

Table IV.3b. Comparisons of Euploids and Aneuploids within High, Low and Unselected Populations.

CHARACTERS	HIGH		LOW		UNSELECTED	
	t-value	P	t-value	P	t-value	P
Plant Height	2.63	0.010	6.42	0.001	7.22	0.001
No. of Tillers	0.40	0.690	1.82	0.073	2.22	0.032
No. of Spikelets	1.35	0.182	2.77	0.007	3.75	0.001
Spike Length	0.77	0.441	2.18	0.034	4.49	0.001
Seed-Set	3.58	0.01	4.23	0.001	3.88	0.001

the "high", the "low" and the unselected populations do not differ in their average performances. The variations in the differences between eu- and aneuploids in the three populations are, therefore, due to the variations of euploid plants. This was indeed observed in table II.1 (Section Two). From the same table (II.1) it was also apparent that there has been a greater effect of partial inbreeding in the high population. As a result the mean values for the vegetative characters of euploid plants were reduced and the differences compared to the aneuploids became relatively small. This suggests that the high population as a whole is more uniform compared to the other two populations. In contrast, the unselected population, being outbred, shows heterosis in the euploid plants which increases the difference from the aneuploids. A similar trend is also found in the low population.

Table IV.4. Comparisons of Aneuploids from High, Low and Unselected Populations.

CHARACTERS	HIGH/LOW		HIGH/UNSELECTED		UNSELECTED/LOW	
	n=71		n=61		n=58	
	t-value	P	t-value	P	t-value	P
Plant Height (cms)	1.28	0.204	0.42	0.674	0.77	0.445
No. of Tillers per plant	0.50	0.616	0.98	0.331	0.59	0.556
No. of Spikelets per spike	0.94	0.351	1.07	0.289	0.19	0.850
Spike Length (cms)	0.40	0.689	0.72	0.471	0.89	0.378
Seed-set	0.13	0.264	0.03	0.976	1.00	0.322

Comparisons of Different Aneuploid Groups

Hagberg & Ellerström (1959) found significant differences in seed-size of hypo- and hyperaneuploids present in tetraploid rye populations and suggested that this may be related to the reduced vigour of the hypoploids as compared to the hyperploids. Levan (1942) also seemed to have obtained similar results in sugarbeet. On the other hand Moore's (1963) results show that under Swedish conditions the fertility levels of 27 and 29-chromosome plants are very similar (52% and 53% respectively) but his Davies materials showed a wider difference. The mean seed-set in 27 and 29 chromosome plants were 56.87% and 61.80% respectively. From these reports it seems that on an average the 29-chromosome plants perform better than 27-chromosome plants and, therefore, from fertility standpoint the presence of hyperaneuploids in a tetraploid population may be more tolerable than hypoaneuploids. To confirm this all the aneuploid plants from the three populations were grouped together according to their chromosome number. At the same time all the euploid plants selected at random from the three populations were added together, thus giving the following classes of plants.

(1) 27-Chromosome Plants

(2) 28-Chromosome Plants

(3) 29-Chromosome Plants

(4) Other Aneuploids.

The mean figures for five morphological characters of the respective classes of plants are presented in table IV.5a and table IV.5b gives their comparisons.

Although the 29-chromosome plants have slightly higher mean values than 27-chromosome plants for all the characters except plant height, the difference is insignificant in each case. Therefore, in the present material the performance of 29-chromosome plants is statistically similar to that of 27-chromosome plants. But the euploids (2n=28) are significantly superior to both 27 and 29-chromosome plants and the difference is even greater for plant height and seed-set when euploids are compared with other aneuploid class. From this one makes the obvious conclusion that aneuploids, in general, are inferior to euploids and there is no clear indication that hyperaneuploids perform better than hypoaneuploids, although there may be differences in seed-sizes (Hagberg & Ellerström, l.c.).

Table IV.5a. Means ± Standard Errors of Morphological Characters of Different Aneuploid Groups.

CHARACTERS	27-Chromosome n=29	28-Chromosome n=95	29-Chromosome n=57	Other Aneuploids n=10
Plant Height (cms)	114.21±3.155	134.42±1.589	114.05±2.621	107.20±6.552
No. of Tillers per plant	5.52±0.488	6.85±0.311	5.54±0.387	6.00±0.745
No. of Spikelets per spike	23.17±0.805	26.98±0.389	24.68±0.528	25.60±1.593
Spike Length (cms)	9.91±0.311	11.29±0.156	10.53±0.259	10.38±0.484
Seed-set (Angular Values)	44.93±2.327	56.15±0.961	47.18±1.569	41.43±4.317

Table IV.5b. Comparisons of Different Aneuploid Groups.

CHARACTERS	28/27 n=124		28/29 n=152		27/29 n=86		28/Other Aneuploids n=105	
	t-value	P	t-value	P	t-value	P	t-value	P
Plant Height	6.01	0.001	7.06	0.001	0.04	0.972	5.11	0.001
No. of Tillers	2.14	0.035	2.61	0.010	0.04	0.967	0.86	0.392
No. of Spikelets	4.57	0.001	3.54	0.001	1.61	0.110	1.06	0.292
Spike Length	4.20	0.001	2.70	0.008	1.45	0.150	1.81	0.073
Seed-set	5.19	0.001	5.16	0.001	0.82	0.416	4.51	0.001

II. Translocation Heterozygotes

Several studies on translocation in autotetraploid rye have been reported (Ahloowalia, 1963; Sybenga, 1966, 1972, 1973). These interchanges were induced artificially at the diploid level and the chromosome number was subsequently doubled. In spite of the possible occurrences of spontaneous translocations in tetraploid populations, as they occur in diploids (Muntzing & Prakken, 1941), until now no spontaneous chromosome interchange at tetraploid level has been reported. This is probably because of the difficulties encountered in identifying an interchange in tetraploid materials without the presence of clear large multivalents, for instance an octavalent at metaphase-I. Sybenga (1973) reports that many translocation heterozygotes in autotetraploids show a significant decrease in larger multivalents, mainly due to preferential pairing. This undoubtedly makes the detection of a spontaneous translocation difficult and tedious.

During the course of the present cytological investigation, chromosome interchanges were detected in eight plants, four from the low population, three from the unselected population used in the comparison trial of 1972 (Section Two). Another interchange was obtained from a different sample of unselected material. The methods of identification for these spontaneous interchange heterozygotes were both direct and indirect. If in a 28-chromosome plant an octavalent is observed at metaphase-I

and the rest of the chromosomes exhibit the pairing pattern expected from an autotetraploid, the detection of an interchange heterozygote is straightforward and direct (see plate IV.1). But in other plants where such a large configuration is absent or not observed in the PMCs analysed at metaphase-I, an indirect method for the identification of interchange heterozygotes has to be adopted. This is discussed below in those cases where octavalent configurations were not observed, even though these plants were, as will be shown, heterozygous for chromosome interchanges.

Tables IV.6a to 6c show the chromosome association in PMCs of three plants in which no octavalent was found. In fact, in Plant No. 72/85-8 no multivalent larger than a pentavalent was found in the PMCs analysed (see Table IV.6b). The PMCs of each plant have been classified into different categories (A to H) according to the various alternatives for chromosome associations. For instance, the chromosome association pattern of the PMCs under category A is possible if the plant was either an eutetraploid (2n=4x) or an interchange heterozygote. On the other hand, the PMCs under category B show chromosome association pattern of a compensated aneuploid (2n=4x+1-1), pentasomic for one chromosome and trisomic for another. But the PMCs of an euploid plant (2n=4x) cannot show the chromosome association pattern exhibited by a compensated aneuploid (2n=4x+1-1) and <u>vice versa</u>. Therefore, the two possibilities cancel one another while the interchange heterozygote accommodates the PMCs under both categories, A and B. By similar reasonings, all other possibilities such as hexasomic-disomic (2n=4x+2-2)

Table IV.6a. Metaphase-I Configurations in PMCs of Plant No. 72/85-5.

PMC Category	MI CONFIGURATIONS I	II	III	IV	V	VI	VII	VIII	No. of PMC	REMARKS
A	2	8	2	1					2	Possible in an euploid (2n=4x) or in a translocation heterozygote
	2	6	2	2					3	
	1	8	1	2					6	
	-	8	-	3					1	
	1	6	1	3					1	
	2	4	2	3					1	
	1	10	1	1					1	
B	-	8	1	1	1				1	Possible in 2n=4x+1-1 or in[a] translocation heterozygote
	-	6	1	2	1				1	
C	-	11	2	-	-				1	Possible in 2n=4x+2-2 or in a translocation heterozygote
	-	9	2	1					2	
	-	3	-	4	-	1			1	
D	1	2	2	3	1				1	Possible in 2n=4x+1-1 or 2n=4x+2-2 or in a translocation heterozygote
	2	7	1	1	1				1	
	2	3	1	3	1				1	
	1	7	-	2	1				1	
E	1	6	2	1	1				1	Possible in 2n=4x+1-1 or in 2n=4x+2-2 or in 2n=4x+3+1-3-1 or in a translocation heterozygote
F	1	8	-	1	-	-	1		1	Possible in 2n=4x+3-3 or in a translocation heterozygote
G	-	-	-	-	-	-	-	-	-	(See table IV.6c.)
H	1	5	3	2					1	Possible in a translocation heterozygote only
	1	3	3	3					1	
	1	9	3						2	
	2	5	4	1					1	
Total	34	320	50	56	7	1	1	-	32	
Mean	1.06	6.88	1.56	1.75	0.22	0.03	0.03	0.00		

Table IV.6b. Metaphase-I Configurations in PMCs of Plant No. 72/85-8

PMC Category	MI CONFIGURATIONS I	II	III	IV	V	VI	VII	VIII	No. of PMC	REMARKS
A	1	8	1	2					3	
	1	6	1	3					1	
	4	3	2	3					1	Possible in 2n=4x
	3	8	3						1	or a translocation
	4	6	-	3					1	heterozygote
	-	8	-	3					1	
	2	9	-	2					1	
	2	6	2	2					1	
	2	8	2	1					2	
	1	4	1	4					1	
B	-	10	1	-	1				1	Possible in 2n=4x+1-1 or in a translocation heterozygote
C	-	9	2	1					2	Possible in 2n=4x+2-2
	-	11	2	-					1	or in a translocation heterozygote
D	2	5	1	2	1				1	Possible in 2n=4x+1-1 or 2n=4x+2-2 or in a translocation heterozygote
H	1	9	3						2	Possible in a translocation heterozygote
Total	27	156	30	30	2	-	-	-	20	
Mean	1.35	7.80	1.50	1.50	0.10	-	-	-		

Table IV.6c. Metaphase-I Configurations in PMCs of Plant No. 39B.

PMC Category	MI CONFIGURATIONS I	II	III	IV	V	VI	VII	VIII	No. of PMC	REMARKS
A	-	8	-	3					3	
	2	7	-	3					1	Possible in an euploid (2n=4x) or in a translocation heterozygote
	3	7	1	2					1	
	-	12	-	1					1	
	4	5	2	2					1	
	-	2	-	6					1	
	2	8	2	1					1	
	1	6	1	3					2	
C	-	9	-	1	-	1			1	Possible in 2n=4x+2-2 or in a translocation heterozygote
	-	7	-	2	-	1			3	
	-	7	2	2					2	
	-	9	2	1					1	
	-	3	-	4	-	1			1	
D	1	5	-	3	1				1	Possible in 2n=4x+1-1 or in 2n=4x+2-2 or in a translocation heterozygote
	1	7	-	2	1				2	
F	1	6	-	2	-	-	1		1	Possible in 2n=4x+3-3 or in a translocation heterozygote
G	2	6	-	2	-	1			1	Possible in 2n=4x+2-2 or in 2n=4x+3-3 or in a translocation heterozygote
H	2	3	4	2					1	Possible in a translocation heterozygote
Total	21	167	17	59	3	6	1	-	25	
Mean	0.84	6.68	0.68	2.36	0.12	0.24	0.04	-		

Table IV.6d. Metaphase-I Configurations in PMCs of Plant No. 72/104-10.

MI CONFIGURATIONS								No. of PMC
I	II	III	IV	V	VI	VII	VIII	
2	6	1	1	-	-	1		2
-	8	-	3					2
-	4	-	5					4
-	6	-	4					3
-	9	-	1					2
1	7	-	2	1				1
2	7	-	3					1
2	5	-	4					1
-	11	-	-	-	1			1
1	2	1	3	-	-	-	1	1
-	7	-	2	-	1			2
-	-	-	5	-	-	-	1	1
-	6	-	2	-	-	-	1	1
2	6	-	2	-	1			1
-	5	-	3	-	1			1
1	6	1	1	-	-	-	1	1
Total 13	149	4	71	1	7	2	4	25
Mean 0.52	5.96	0.16	2.84	0.04	0.28	0.08	0.16	

Table IV.6e. Metaphase-I Configurations in PMCs of Plant No. 72/142-10

MI CONFIGURATIONS								No. of PMC
I	II	III	IV	V	VI	VII	VIII	
-	6	1	2	1				1
1	8	1	2					1
-	6	-	4					4
1	7	-	2	1				2
-	7	2	2					2
-	6	1	3					2
1	9	-	1	1				2
-	10	-	2					2
1	4	1	4					1
-	5	-	3	-	1			1
-	6	-	2	-	-	-	1	1
1	3	1	3	-	1			1
Total 9	134	10	52	5	2	-	1	20
Mean 0.45	6.70	0.50	2.60	0.25	0.10	0.00	0.05	

Table IV.6f. Metaphase-I Configurations in PMCs of Plant No. 72/28-4.

MI CONFIGURATIONS								No. of PMC
I	II	III	IV	V	VI	VII	VIII	
-	2	-	4	-	-	-	1	1
2	7	1	1	1				1
-	6	1	2	1				2
1	9	3						1
2	7	-	3					2
1	4	2	2	1				1
1	7	-	2	2	1			1
-	12	-	1					1
3	6	3	1					1
1	8	1	2					1
1	4	-	3	-	-	1		1
-	9	-	1	-	1			1
1	6	1	3					2
-	8	-	1				1	1
-	8	-	3					1
-	5	-	3	-	1			1
-	8	1	1	1				1
Total 16	135	15	41	6	2	1	2	20
Mean 0.80	6.75	0.75	2.05	0.30	0.10	0.05	0.10	

Table IV.6g. Metaphase-I Configurations in PMCs of Plant No. 72/28-5.

MI CONFIGURATIONS								No. of PMC
I	II	III	IV	V	VI	VII	VIII	
-	5	-	3	-	1			1
2	8	2	1					1
-	6	1	2	1				1
-	7	-	2	-	1			1
-	8	1	1	1				1
-	10	-	-	-	-	-	1	1
1	4	2	2	1				1
-	4	3	1					1
-	8	1	1	1				1
-	8	-	3					1
Total 3	68	10	16	4	2		1	10
Mean 0.30	6.80	1.00	1.60	0.40	0.20	-	0.10	

or heptasomic-monosomic (2n=4x+3-3) condition can be proved invalid, even if such plants are considered viable. Therefore, the only alternative which is left open and satisfies the chromosome association pattern under each category of PMCs is/an interchange heterozygote. The nullisomic condition (2n=2x+4-4) can be ruled out straightaway because such plants are least likely to survive when the chromosome complement represents an autotetraploid.

Tables IV.6d to 6g show chromosome associations in PMCs of four plants in which octavalent configurations in varying numbers appeared. The presence of an octavalent in a 28-chromosome plant would suggest an interchange heterozygote. Furthermore, the chromosome association pattern in/the PMCs without an octavalent can also be shown to be possible only if the plant was an interchange heterozygote.

Table IV.6h presents the M-I configurations of plant No. 72/36-11 which was an aneuploid (2n=29) and at the same time it was an interchange heterozygote. The chromosome association pattern in PMCs shown in the table suggests that the additional chromosome was not involved in the translocation complex (see also Plate No. IV.1, figs. k&l).

Table IV.6h. Metaphase-I Configurations in PMCs of Plant No. 72/36-11 (2n=29).

M-I CONFIGURATIONS								No. of PMC
I	II	III	IV	V	VI	VII	VIII	
1	6	-	2	-	-	-	1	1
1	4	1	3	1				1
-	5	-	2	1	1			3
-	4	-	4	1				1
-	7	1	3					2
-	7	1	1	-	-	-	1	3
-	6	3	2					1
1	6	2	1	-	1			1
1	3	2	2	-	-	-	1	1
-	3	2	3	1				1
-	7	-	1	1	1			2
-	6	1	2	-	1			3
1	7	-	2	-	1			1
2	6	3	-	-	1			1
-	10	1	-	-	1			1
2	6	1	3					1
-	3	-	3	1	1			1
Total 9	146	23	48	9	13	-	5	25
Mean 0.36	5.84	0.92	1.92	0.36	0.52	-	0.20	

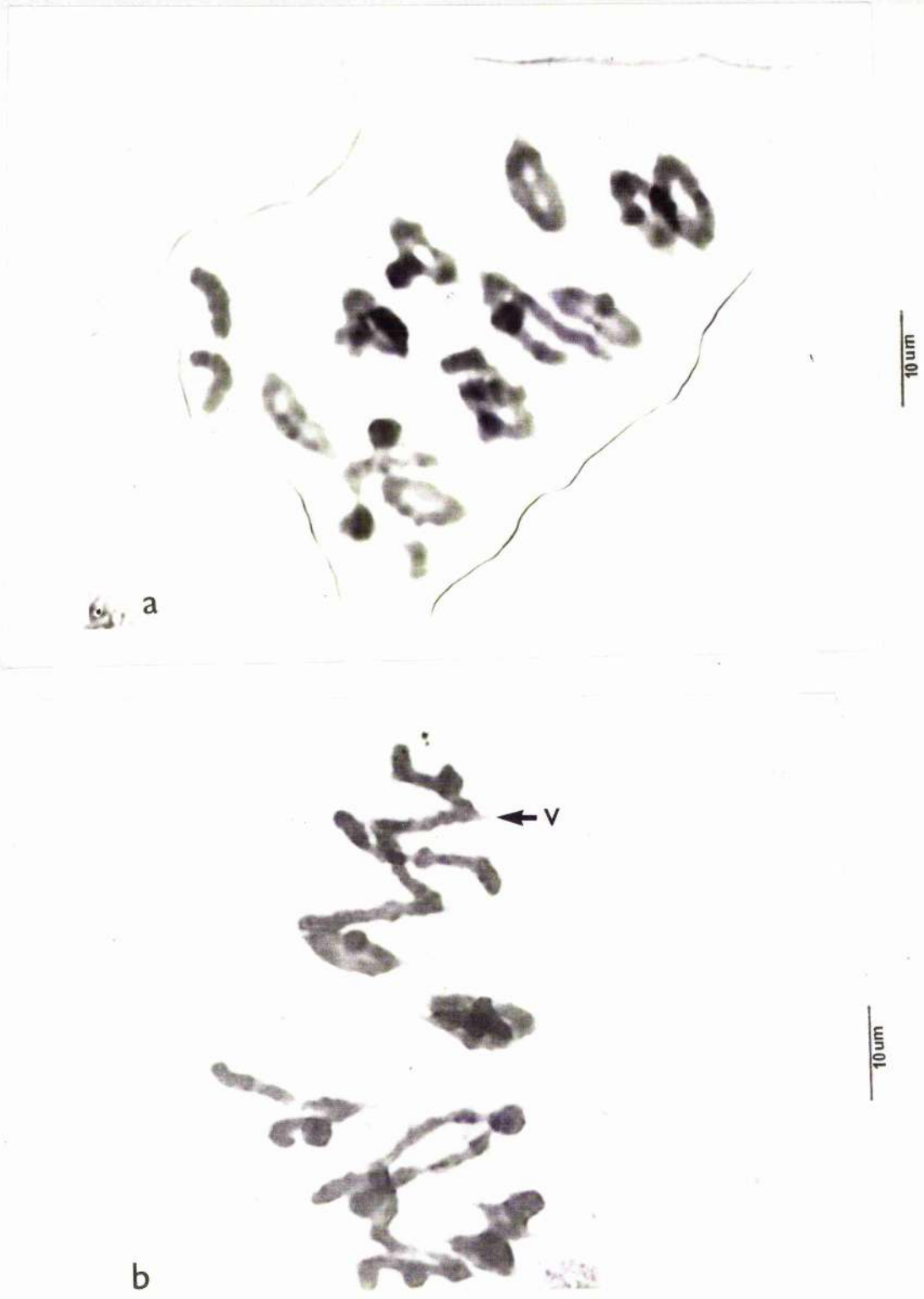


PLATE IV.1. Chromosome Associations in Translocation Heterozygotes

Plant No. 72/85 - 5 ($2n = 28$)

a - $2^{I}\ 6^{II}\ 2^{III}\ 2^{IV}$; b - $6^{II}\ 1^{III}\ 2^{IV}\ 1^{V}$

c

10 µm

d

10 µm

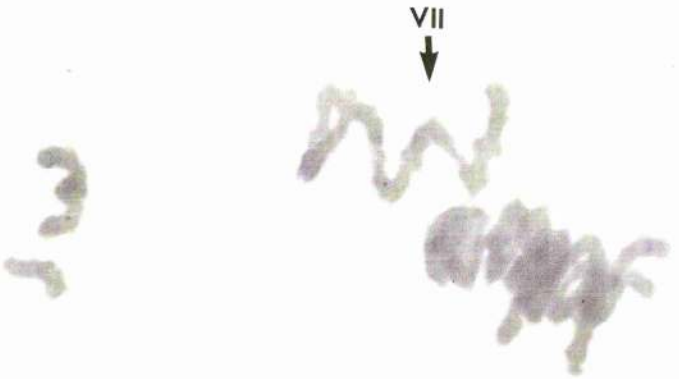
PLATE IV.1. Translocation Heterozygotes (contd.)

Plant No.,39B (2n = 28)

c - $9^{II}\ 2^{III}\ 1^{IV}$; d - $2^{I}\ 3^{II}\ 4^{III}\ 2^{IV}$

e

10µm



f

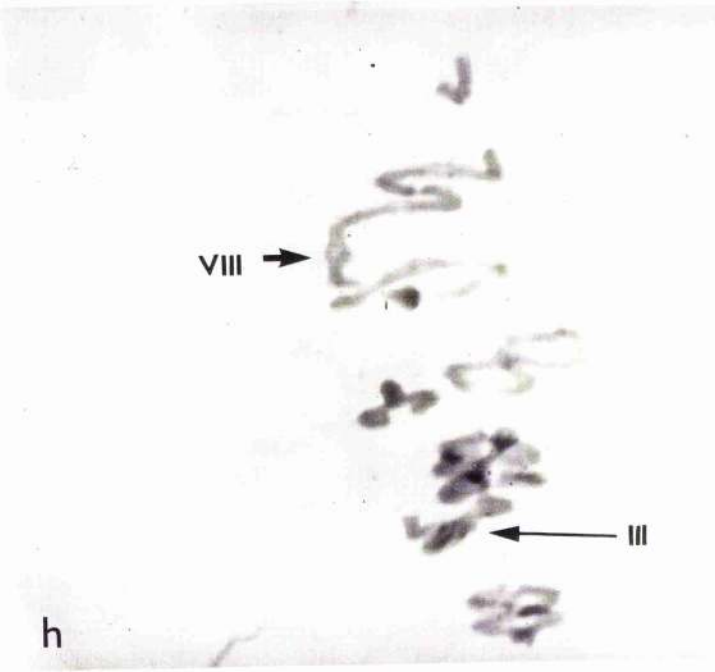
10µm

PLATE IV.1. Translocation Heterozygotes (contd.)

Plant No. 393 (2n = 28)

e - $7^{II}\ 2^{IV}\ 1^{VI}$; f - showing 1^{VII} & 1^{I}

10 um



10 um

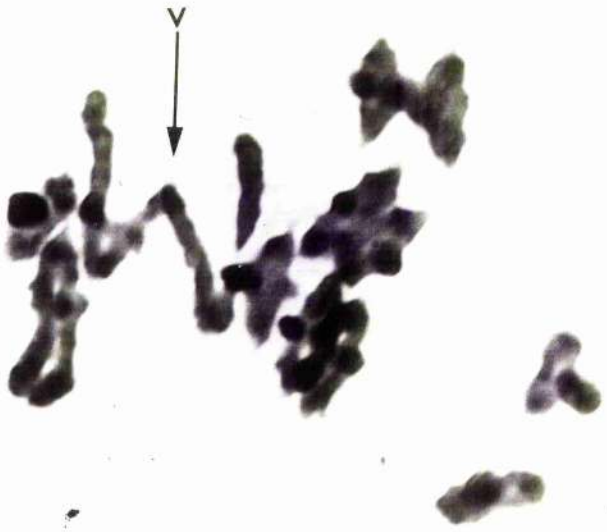
PLATE IV.1. Translocation Heterozygotes (contd.)

Plant No. 72/104 - 10 (2n = 28)

g - $_{7}^{II}$ $_{2}^{IV}$ $_{1}^{VI}$; h - $_{1}^{I}$ $_{2}^{II}$ $_{1}^{III}$ $_{3}^{IV}$ $_{1}^{VIII}$

j

10 µm



i

10 µm

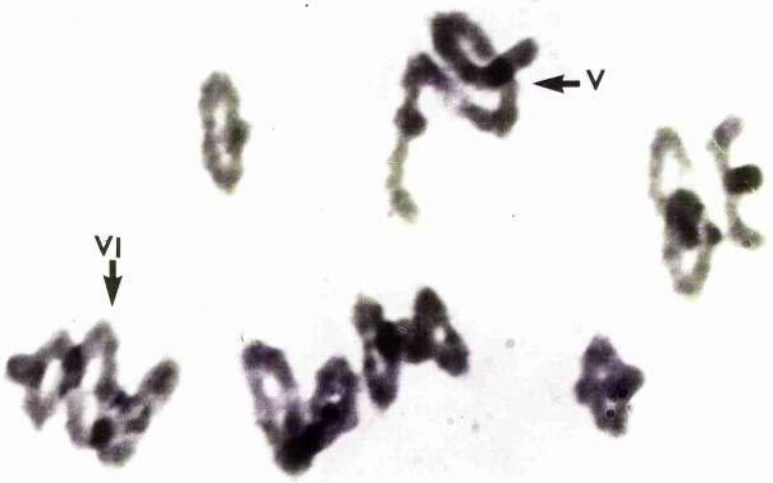
PLATE IV.1. Translocation Heterozygotes (contd.)

Plant No. 72/28 - 5 (2n = 28)

i - $6^{II}\ 1^{III}\ 2^{IV}\ 1^{V}$;

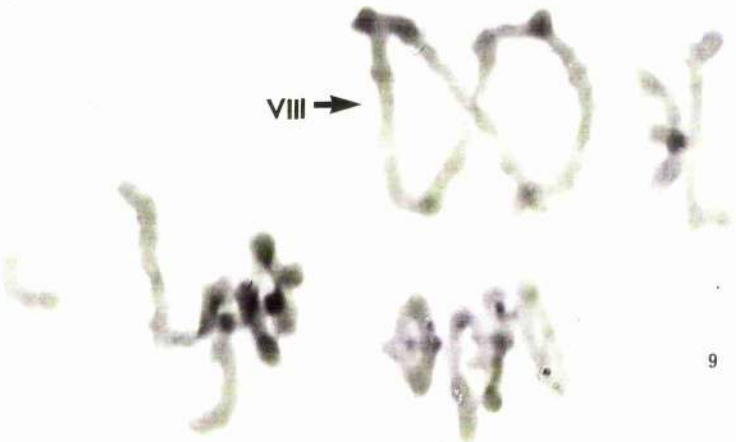
Plant No. 72/28 - 4 (2n = 28)

j - $2^{II}\ 4^{IV}\ 1^{VIII}$



k

10 µm



9

l

10 µm

PLATE IV.1. Translocation Heterozygotes (contd.)

Plant No. 72/36 - 11 (2n = 29)

k - $7^{II}\ 1^{IV}\ 1^{V}\ 1^{VI}$; l - $1^{I}\ 6^{II}\ 2^{IV}\ 1^{VIII}$

III. Anaphase Bridge with or without Fragments due to Error in Crossing-Over.

It is now well established that the presence of a dicentric bridge and an acentric fragment at anaphase-I is inadequate and sometimes even invalid for the previously accepted indication of a paracentric inversion. Brdiges with or without fragments may also arise from sister and/or non-sister chromatid breakage and reunion (see Rees, 1955; Rees & Thompson, 1955; Wilson, et al, 1959; Lewis & John, 1966; Jones, 1968). Such errors may occur in natural populations (John, et al, 1960; Lewis & John, 1966), in species hydrids (Walters, 1952; Jones, 1968), by enforced inbreeding in cross-pollinated species (Rees, 1955; Rees & Thompson, 1955) or by irradiation (Wilson, et al, 1959). The anomalous cell-types, observed in the present study, arising out of breakage and reunion can be classified as follows:

(1) Dicentric Chromatid Bridges with Acentric Fragments:

This type of anomalous cell occurs rarely and if more than one cell is observed, the size of the fragment and of the bridge may vary because different chromosomes can be involved in different cells. The PMC shown in Plate No. IV.2 was the single abnormal cell observed among several hundreds of PMCs (from the three anthers) of the plant. In a few other plants one or more anaphase-I cells, with dicentric bridges and acentric fragments, were observed,

Plate IV.2. Dicentric Chromatid Bridge with Acentric Fragment

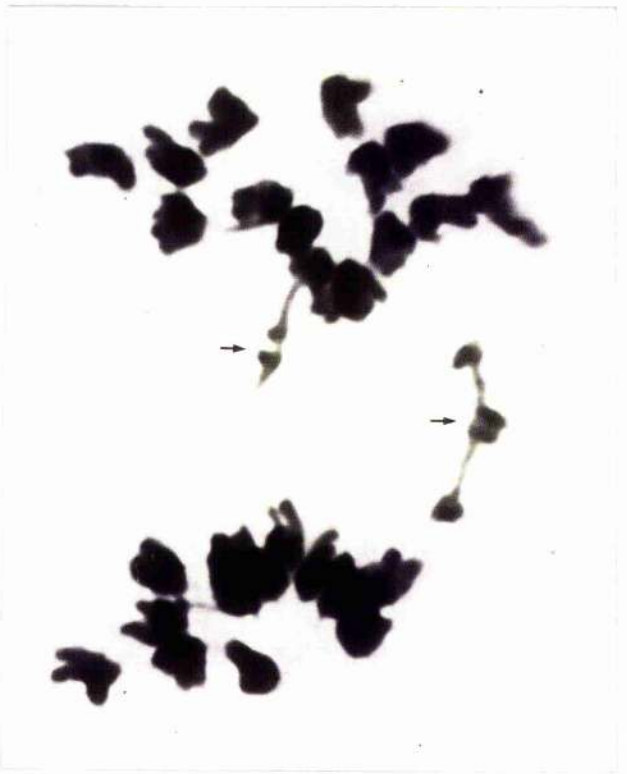


PLATE IV.3. Side-Arm Bridges

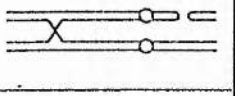
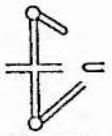
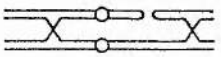
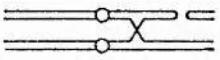
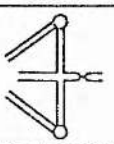
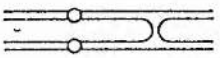
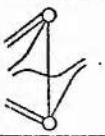
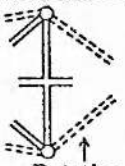
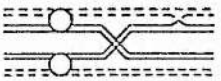
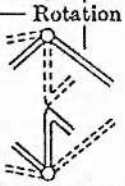
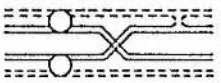
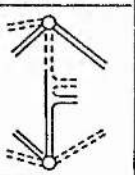
	Type	Exchange pattern	A—I Configuration	T—I Consequences
Chromatid-breakage	1. 	Iso-locus sister-chromatid union in a non-chiasmate arm		Free chromosome fragment
	2. 	Iso-locus sister-chromatid union between the centromere and a chiasma		± Free fragment depending on how early the acentric is released
	3. 	Iso-locus sister-chromatid union beyond a chiasma		Dicentric chromatid bridge plus an acentric chromatid fragment
	4. 	Iso-locus breakage of non-sister chromatids with inverted union		Dicentric chromatid bridge plus an acentric chromatid fragment
Half-chromatid breakage	1. 	Half-chromatid exchange between non-sister chromatids		Normal ?
	2. 	Non-sister, half-chromatid exchange between sister chromatids & beyond a chiasma		False dicentric half-chromatid bridge with two converging side arms
	3. 	Non-sister half-chromatids union between sister chromatids & beyond a chiasma		True dicentric half-chromatid bridge with two parallel side arms

Fig. IV.1. Consequences of Chromatid Breakages and Re-unions (after Lewis & John, 1966).

but in no case the frequency of the aberrant anaphases was high, in fact, it was much less than 1%. However, some genotypes which are prone to breakage and reunion can show quite a high frequency of anaphase bridges and fragments (see Jones, 1968). In the present material, however, such an anomaly occurred very rarely, apparently by accident.

(2) Side-Arm Bridges:

Like dicentric bridges and acentric fragments, side-arm bridges also result from errors in crossing-over. One such PMC is shown in Plate No. IV.3. It will be noted that the side-arm bridge is not usually associated with a fragment. Two kinds of side-arm bridges have been described by Wilson (1959), one is false and the other is true (see figure IV.1). Apparently the false side-arm bridges can eventually lead to the separation of the chromosomes, whereas in the case of the true arm bridge, there must be a break somewhere between the side arm and the centromere in order to complete the first anaphase separation.

IV. A Case of Spontaneous Paracentric Inversion.

As pointed out above, the presence of a bridge and a fragment at anaphase-I is not enough for the indication of a paracentric inversion. For the confirmation of a paracentric inversion it is, therefore, desirable to identify that the bridge and the fragment involve the same chromosome pair(s), the size of the fragment is constant and also it is desirable to identify the typical loop formation at meiotic prophase.

In the present case it was not possible to identify the particular chromosome involved in the inversion because, firstly, no mitotic karyotype analysis could be accomplished and, secondly, all the rye chromosomes, being very similar in length and centromeric positions, make it more difficult to identify the inversion from chromosome morphology. Attempts to find a loop formation at pachytene were not successful because the chromosomes took a very long tortuous course at this stage and it was extremely difficult to follow the paired chromosomes. However, the size of the acentric fragment appeared to be reasonably constant (see figures in Plate IV.4) and each of the five theoretically possible anaphase-I configurations (McClintock, 1938; Lewis & John, 1963) was observed (see Plate IV. 4). The origin of these configurations has been demonstrated in figure IV.2. . From these it could be concluded with confidence that the case was a paracentric inversion.

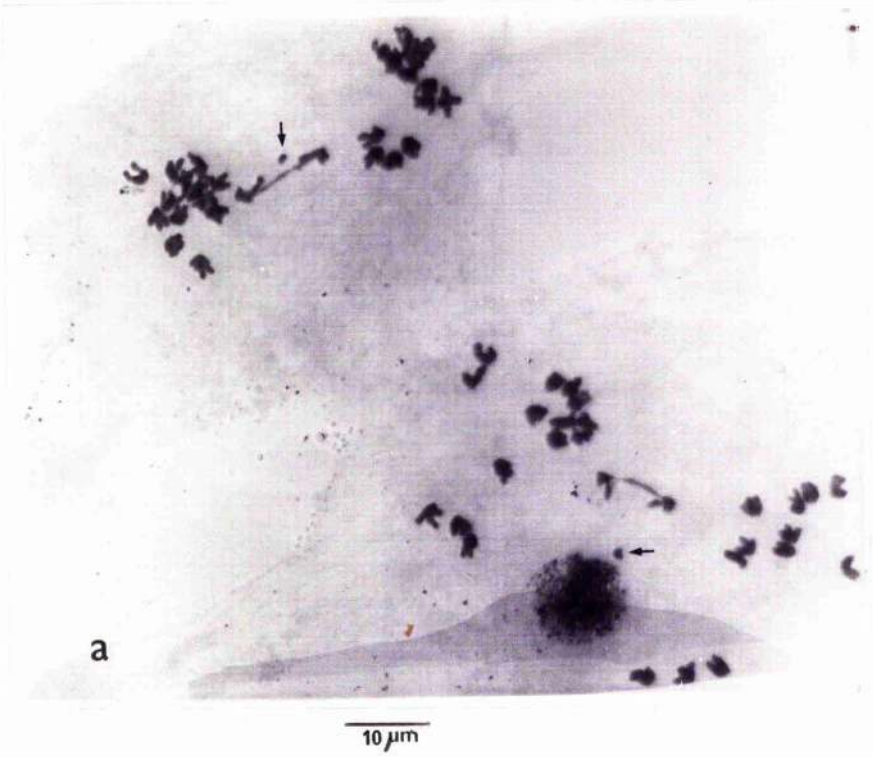


PLATE IV.4. Anaphase-I in an Inversion Heterozygote

a - Two adjacent PMCs each showing a bridge plus a fragment (BF).

b - One fragment without a bridge. This can arise from 3-strand double cross-over forming a loop plus a fragment(LF).

PLATE IV.4. Inversion Heterozygote (contd.)

c - Anaphase-II with a bridge plus a fragment. Arising from loop plus fragment at Anaphase-I (IF).

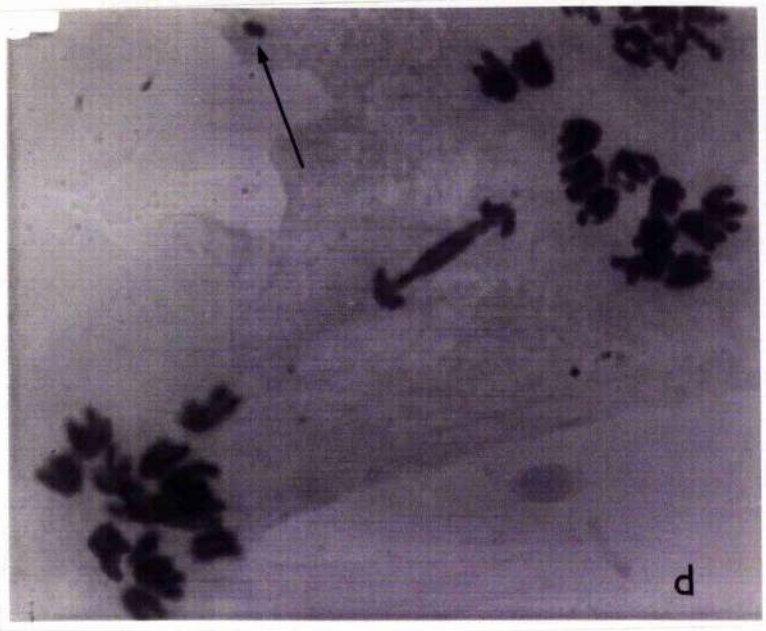


PLATE IV.4. Inversion Heterozygote (contd.)

d - Anaphase-I with double bridges plus one fragment. Another fragment apparently has been lost during squashing.

e - Anaphase-I with double bridges plus double fragments (REFF). This can arise from 4-strand double cross-over.

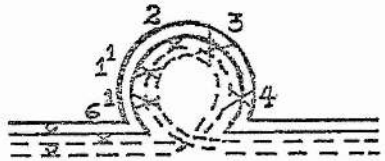
f

PLATE IV.4. Inversion Heterozygote (contd.)

f - Anaphase-II with double bridges plus one fragment. Another fragment apparently has been lost during squashing. This can arise from 4-strand double cross-over forming two loops and two fragments (IIIIF) at anaphase-I.

It could be argued that since the inversion was detected in an autotetraploid individual, the above scheme in figure IV.2, based on inversion heterozygote in a diploid, is not entirely satisfactory. This warrants a consideration of the consequences of an inversion heterozygote in duplex condition. That is, two of the four homologous chromosomes could be inverted in an identical way while the other two chromosomes were normal. Such a case may arise from the fusion of a diploid gamete, which is homozygous for the inversion, with a normal diploid gamete. The pairing behaviour in such an inversion heterozygote when it forms two bivalents or a quadrivalent is demonstrated in figure IV.3 a&b.

Taking into account all the possible ways of chromosome association shown in the above figure, the number of bridges and fragments will vary from 0 to 4. Furthermore, some of the association types will give rise to two separate bridges in the same PMC. In the present case no PMC with two separate bridges was observed nor was there any PMC with 4 bridges + 4 fragments. Absence of these configurations at anaphase-I eliminates the possibility of a duplex paracentric inversion. In concluding so, one must not forget that there may be preferential pairing for the two inverted and the two normal chromosomes. However, the configurations observed and presented in Plate IV.4 would be expected if the inversion in question was present just in one of the four homologous chromosomes. Whether this inverted chromosome is involved in a multivalent formation or not, the anaphase-I configurations will be the same as with a paracentric inversion in a diploid.



		NO C.O. AT 6		CO. AT 6		
	AI AII	SINGLE C.O. WITHIN THE INVERSION AT	DOUBLE C.O WITHIN THE INVERSION AT	SINGLE C.O. WITHIN THE INVERSION AT	DOUBLE C.O. WITHIN THE INVERSION AT	BALANCED CHROMATIDS %
N			1 + 1¹ 2 STRAND DOUBLE		1 + 1¹ 2 STRAND DOUBLE	100 100 100
DF		1 2 3 4	1 + 2 1 + 3 2 + 4 3 + 4 3 STRAND DOUBLE	1 4	1 + 2 2 + 4 3 STRAND DOUBLES	50 50 50 50
DFFF			1 + 4 2 + 3 4 STRAND DOUBLES		1 + 4 4 STRAND DOUBLE	0 0
IF				2 3	1 + 3 3 + 4 3 STRAND DOUBLES	50 50
IFFF					2 + 3 4 STRAND DOUBLE	0

Fig. IV.2. Five Types of Meiotic Anaphase Configurations in a Paracentric Inversion Heterozygote. The positions of various crossing-over are indicated in the inversion loop and one cross-over at proximal region (at 6). N = Normal Anaphase; B = Bridge; ' = Paracentric Loop (after Ekbern, 1974).

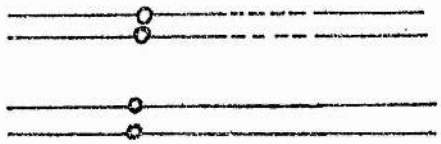
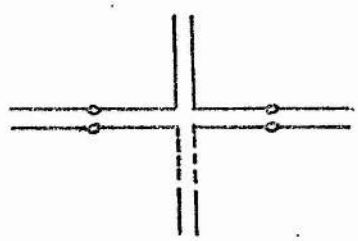
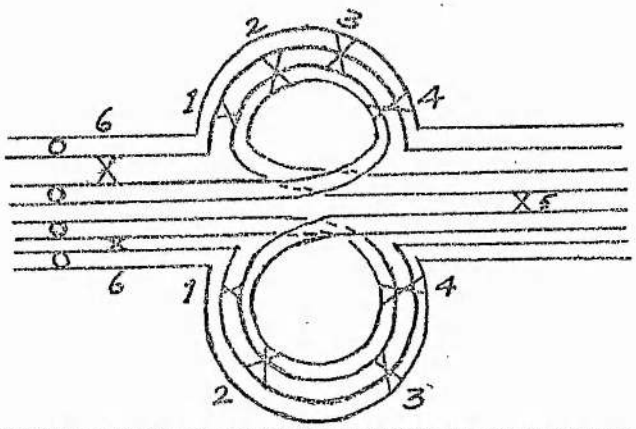
CONFIGURATION	PACHYTENE	CONSEQUENCE AT AI
BIVALENT PAIRS	 A	NORMAL
	  B	With a single or 3-strand double C.O. within both loops:- TWO BRIDGES & TWO FRAGMENTS (see fig-IV.2 for details)
QUADRIVALENT	 A (see B overleaf)	NORMAL

Fig. IV.3a. Chromosome associations in a Duplex Paracentric Inversion Heterozygote. (contd.)

PACHYTENE

QUADRIVLENT

B



	NO C.O. AT 6		C.O. AT 6	
	SINGLE C.O. WITHIN THE INVERSION AT	DOUBLE C.O. WITHIN THE INVERSION AT	SINGLE C.O. WITHIN THE INVERSION AT	DOUBLE C.O. WITHIN THE INVERSION AT
AI AII	1 OR 2 OR 3 OR 4	(1+2) OR (2+4) OR (1+3) OR (3+4)	1 OR 4	(1+2) OR (2+4)
		(1+4) OR (2+3)		
			2 OR 3	
				(2+3)

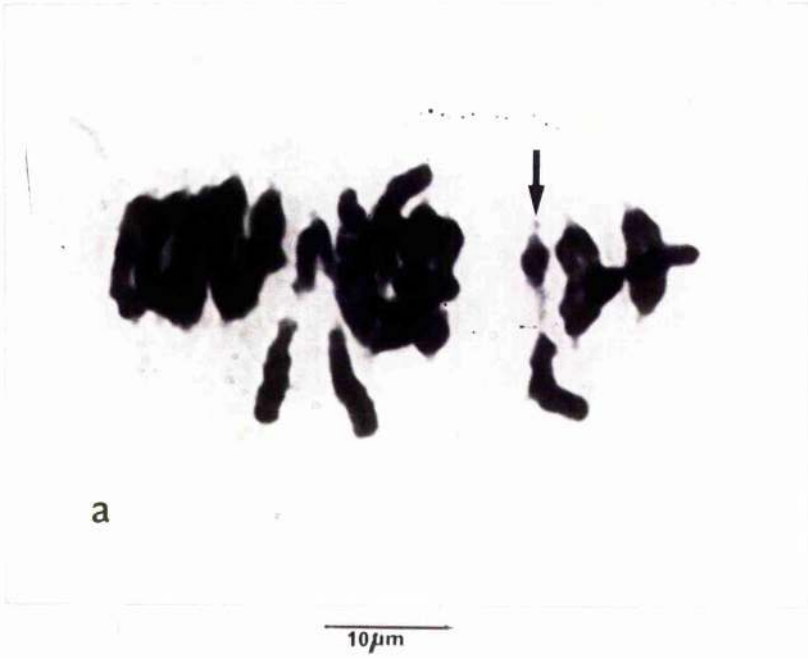
Fig. IV.3b.

V. Centric Fragment

Centric fragments were observed in two plants. In both cases the fragment was often found to be associated end-to-end with its normal homologue, thus forming heteromorphic configurations at metaphase-I (see Plate IV.5). At anaphase-I and II the fragments behaved like a normal chromosome insofar as anaphase separation and movement were concerned. It, therefore, appears that the fragments in both cases were deleted chromosomes as distinct from supernumerary or B-chromosomes.

The fragment occurred spontaneously in both cases and, therefore, their origin is not certain. Darlington (1965) suggested that fragments can occur in both mitotic and meiotic phases, during resting stage or during prophase at meiosis. Whenever in the cell-cycle this may occur, it is evident that one or more breakages along the chromosome length will be necessary to obtain such fragments.

With regard to the homology between fragment and its normal counterparts, it is evident that there is a strict homology at the centromeric region. In addition the end-to-end association of the fragment with its normal counterparts suggests that the fragment may also be homologous at least at one end. On the other hand, end-to-end pairing may be possible even if the chromosome ends are not essentially homologous. Because the centromeric region being homologous, chiasma formation in this



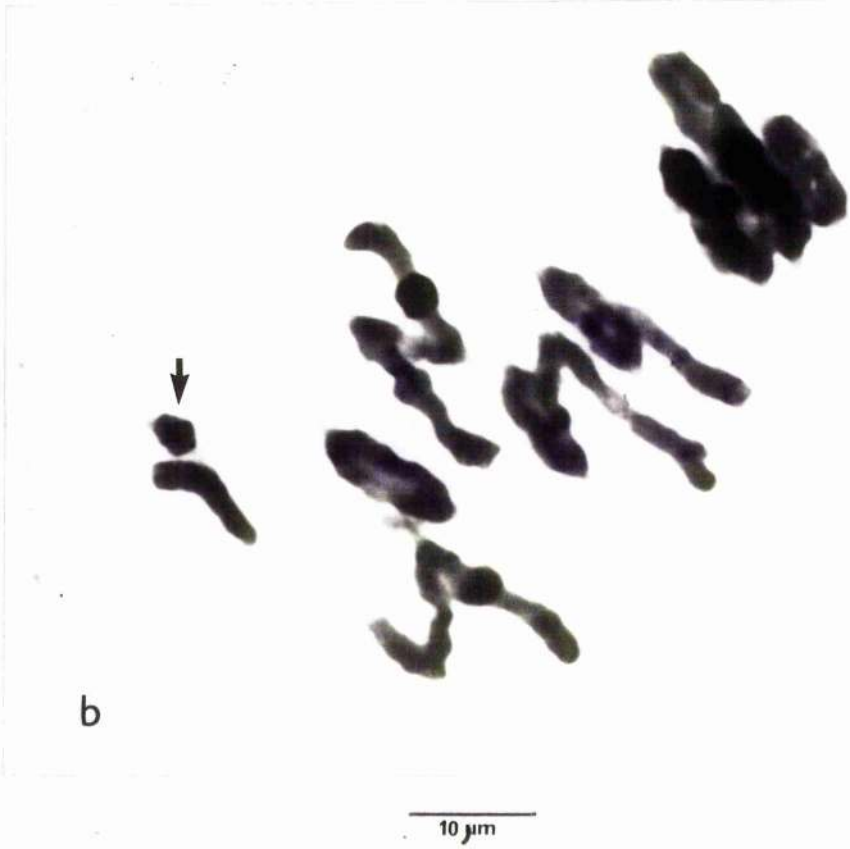


PLATE IV.5. Centric Chromosome Fragment

a - The centric fragment paired with its normal homologue forming a heteromorphic rod-bivalent.

b - The centric fragment lying unpaired.

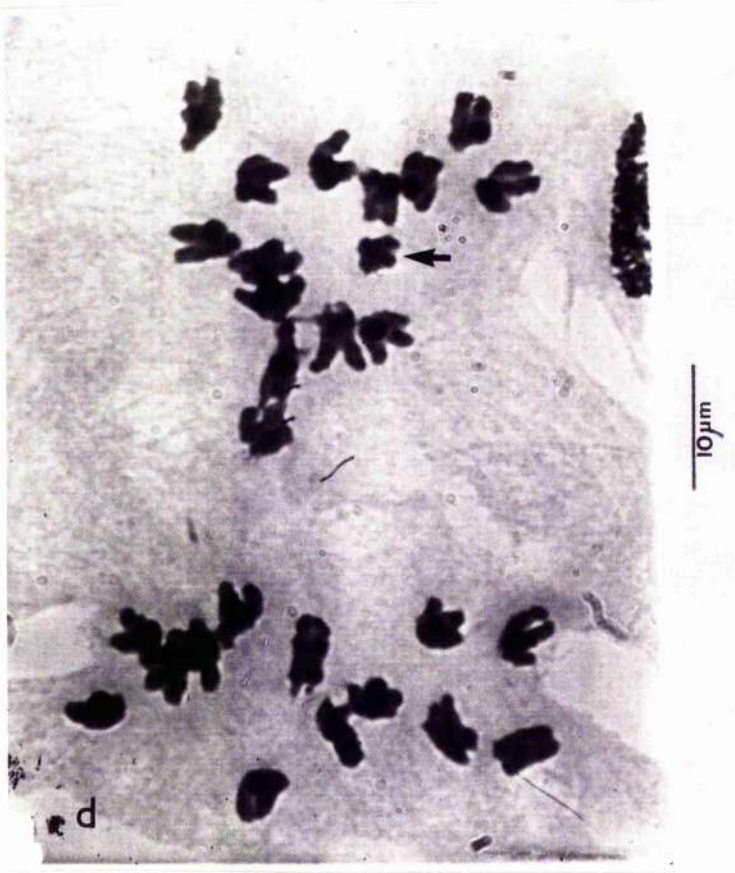


PLATE IV.5. Centric Chromosome Fragment (Contd.)

c - The centric fragment is separating from its normal homologue at A-I.

d - The centric fragment passed on to one pole at A-I.

PLATE IV.5. Centric Chromosome Fragment (contd.)

e - Chromatid separation of the unpaired centric fragment at A-I. Also note the chromatid separation of apparently normal unpaired chromosome.

region is quite feasible and with complete terminalisation of chiasma, the fragment may remain associated at one end only (Darlington, 1965). However, the centric fragment as observed here may play an important part in chromosomal evolution and accelerate the diploidisation process in autopolyploids.

VI. Neo-Centric Activity.

Neo-centric activity in rye has been reported by many workers. The phenomenon is characterised by active movement of certain chromosome ends. Kattermann (1939) seems to be the first to observe such phenomenon in inbred rye. Later Prakken & Muntzing (1942), Östergren & Prakken (1946) and Rees (1955) reported such activity also in inbred rye materials. These authors agree that mainly the short arm of the chromosomes show such activity. Jones (1969) also reported such phenomenon in selfed progenies of Secale dighoricum x S. turkestanicum.

Page - 206
Para 1. Line 5
Oestergren → Ostergren

In the present material neo-centric activity was observed quite often. Plate IV.6 shows PMCs with such activity. The cells in a and c were from the same plant.

The cause of neo-centric activity is not well understood. Rhoades (1952) suggested that the diffusion of centric substances to the ends of the chromosomes induces such activity. Darlington (1965) states that this substance is a diffusible enzyme, passed along the chromosome from the centromere to the ends by a canalised movement and possess the same capacity for generating spindle fibres as the centromere itself. Lewis & John (1963) believe that there may be a correlation between the number of heterochromatic knobs (H-knobs) at chromosome ends and the degree of neo-centric activity. In the

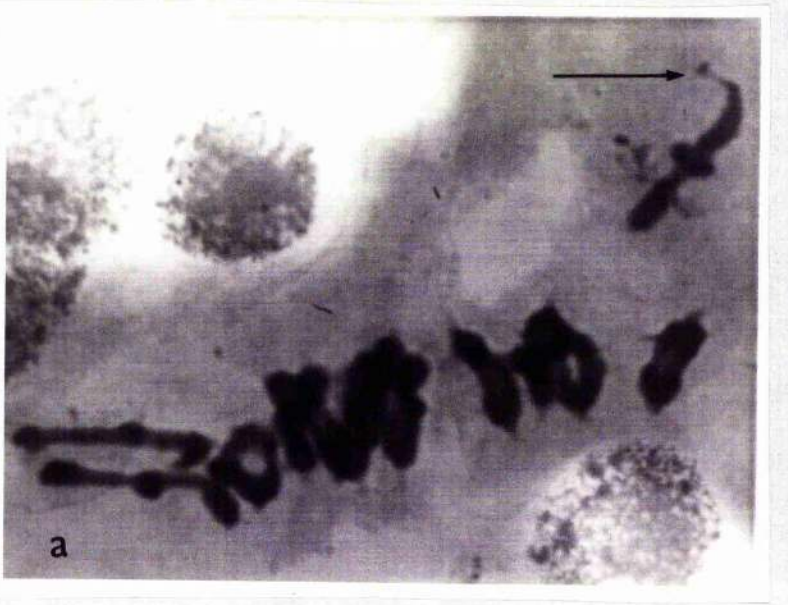
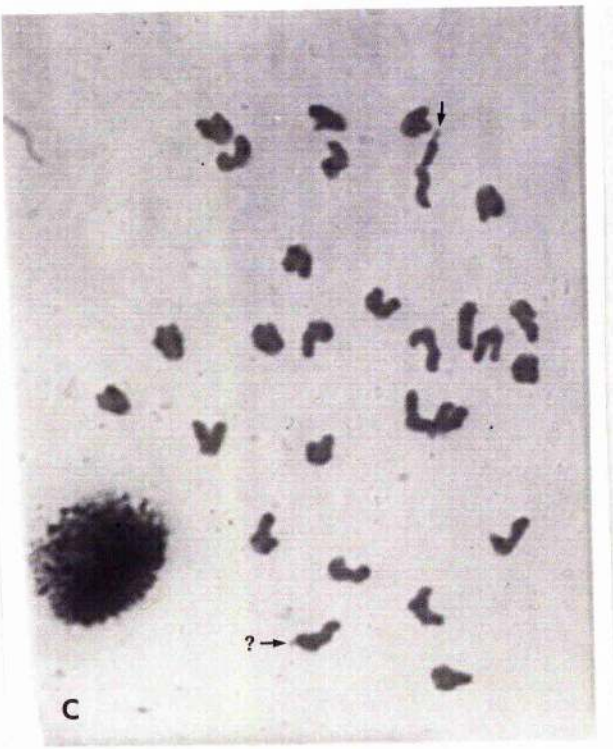


PLATE IV.6. Neo-centric Activity of Chromosomes

a - Metaphase-I with a mobile end (neo-centric) of a rod-bivalent (arrow).

b - Anaphase-I showing neo-centric activity (arrows).



10μm

PLATE IV.6. Neo-centric Activity (contd.)

c - Anaphase-II showing neo-centric activity (arrows).

rye complement there are six H-knobs in the shorter arms and Rees (1955) observed as many as five mobile ends in one PMC. However, usually one pair of chromosomes have been reported to show the phenomenon. Lewis & John (1963) further state that larger knobs have greater neo-centric activity, because the substance secreted by the centromere accumulates near the knob either because the knob acts as a physical barrier or because it actively attracts and absorbs this substance and a larger knob may be more effective in these directions (see also Rhoades, 1952). However, there is evidence from maize that knobless arms may also show neo-centric activity (Rhoades, 1952). But when they do so, the spindle fibres are not developed until anaphase movement has been triggered by the true centromere; in contrast, in the knobbed arms the neo-centric spindles are evident at first anaphase.

VII. Polyard Formation.

At tetrad stage of meiosis, the normal sequences of cell division give rise to four microsporocytes. In a few plants, which were otherwise normal, more than four microsporocytes (polyads) were observed at this stage of meiosis (see Plate IV. 7).

These polyads may have arisen either from multipolar chromosome separation followed by cytokinesis (see Darlington & Thomas, 1937), or from occasionally present asynaptic cells (Plate IV.7, figs. a&b). In the asynaptic cells the chromosomes fail to orient properly on the metaphase-I equatorial plate so that the polarity of the loosely formed configurations or the unpaired chromosomes is not "compact" and convergent. As a result there may be more than two groups of chromosomes following anaphase-I, each group forming a cell wall around it at telophase-I and thus giving raise to the similar consequences as in the case of multipolar chromosome separation. Since the frequency of asynaptic cells and polyads, in otherwise normal plants, is extremely low, the abnormality does not seem to have any serious consequence on fertility.

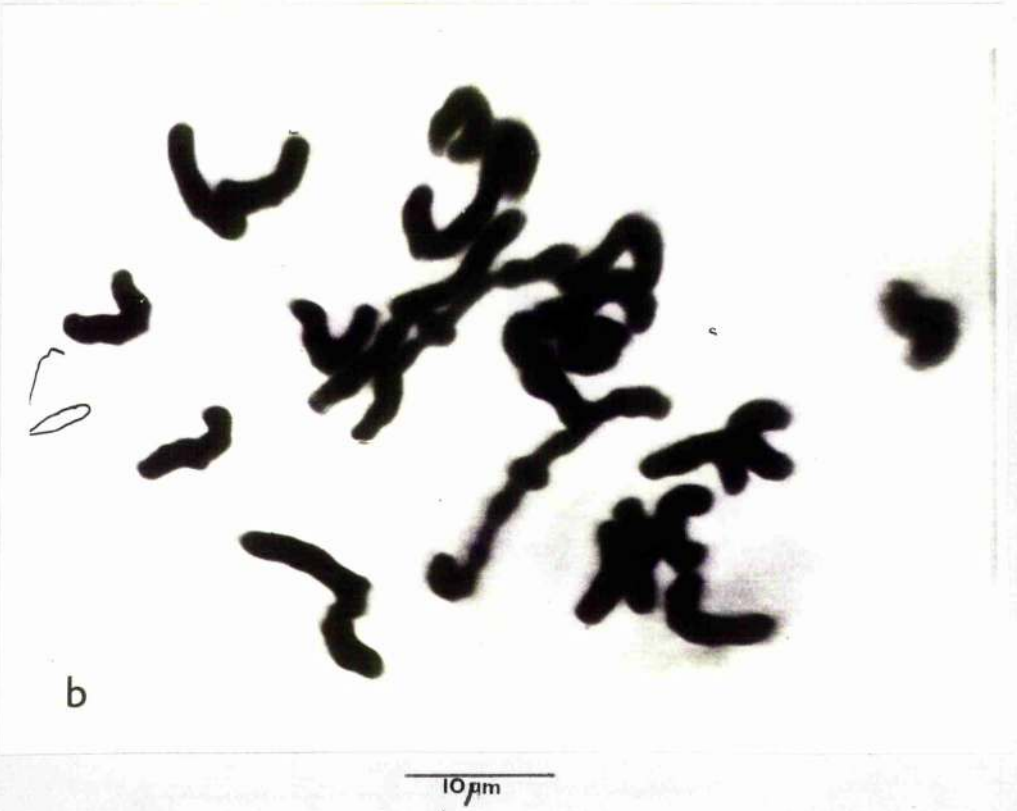
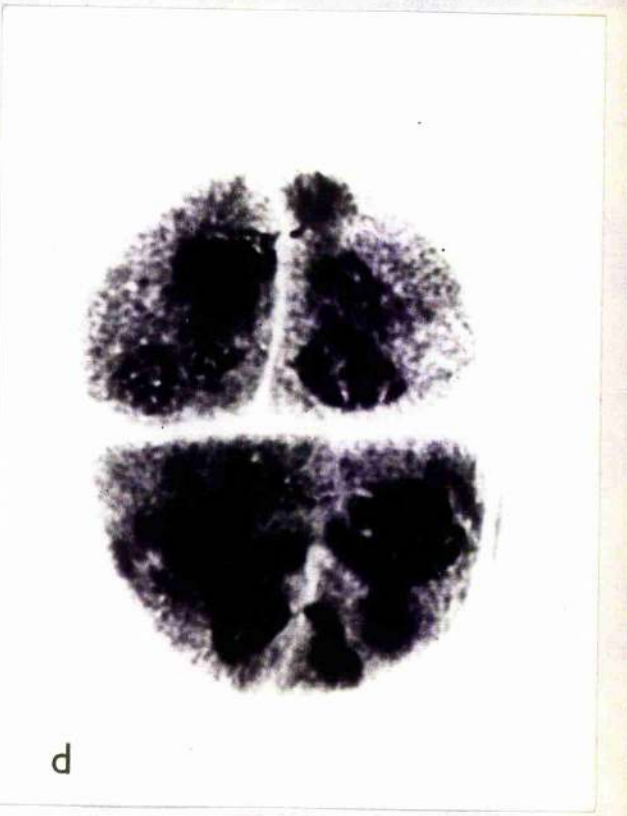
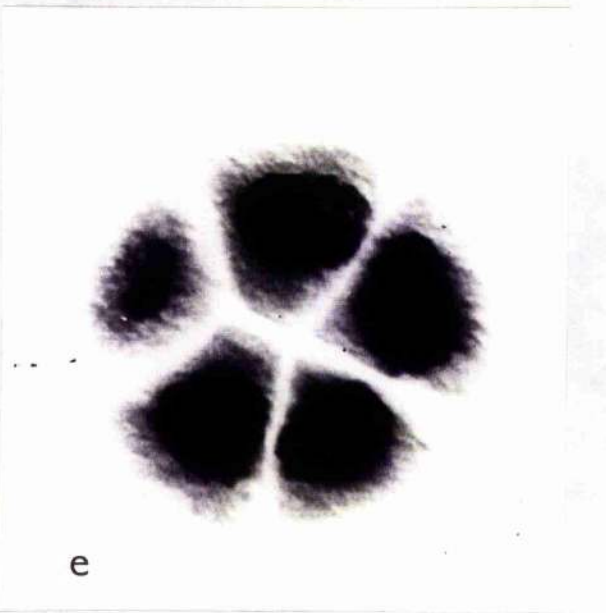


PLATE IV.7. Asynaptic Cells

a & b - Asynaptic cells at metaphase-I. Note very few chiasma formations, clearly visible in figure b.





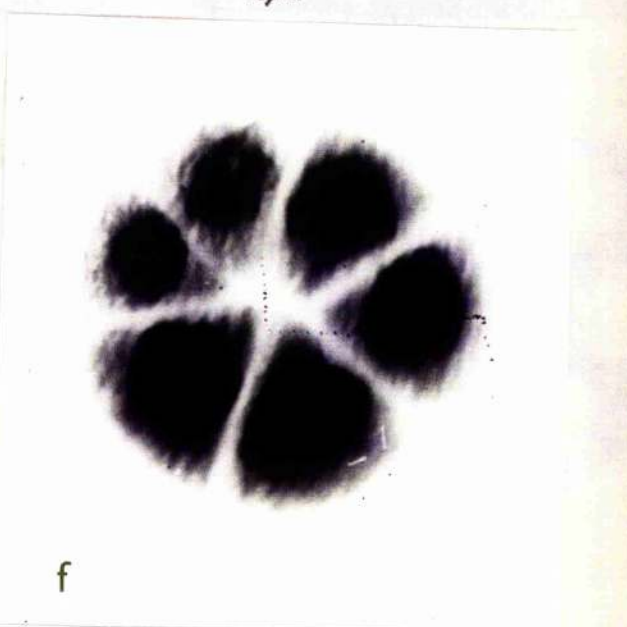


PLATE IV.7. Asynaptic Cells (contd.)

c - Anaphase-I in an asynaptic cell. Note separation of chromosomes and chromatids resulting from paired and unpaired chromosomes of metaphase-I respectively.

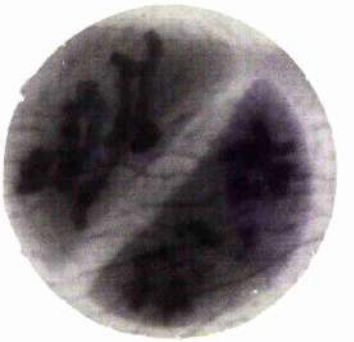
d - Tetrad stage showing numerous micronuclei of verying sizes, presumably arose from an asynaptic cell.

e & f- Polyad formations at tetrad stage.

VIII. A Case of Drastic Abnormality in Cell Division.

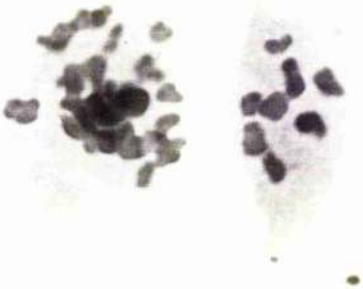
The co-ordination between nuclear division or karyokinesis and cytoplasmic division or cytokinesis is an essential phenomenon involved in the normal process of cell-division. Lack of co-ordination of the two processes or a mistake in any one of them can lead to abnormal end-products. During the course of the present investigation, one plant was observed in which a large number of PMCs at tetrad stage indicated errors of karyokinesis as well as cytokinesis. The PMCs are shown in Plate IV.8.

Unfortunately, earlier stages of meiosis were not available for investigations. Nevertheless, from the figures in Plate IV.8, it is evident that the errors were involved in spindle mechanism as well as cell-wall formation. Figure *b* indicates that anaphase-I separation was incomplete so that the two daughter cells at dyad stage received considerably variable number of chromosomes. At anaphase-II, the unequal dyads seemed to have divided in a majority of cases while in others there was a lack of synchronisation between the two dyad cells. When this happened, usually the daughter cell with higher number of chromosomes showed delayed action or failure of chromosome separation (see figure c in Plate 8). Non-synchronisation at anaphase-II was reported by Darlington & La Cour (1952) in radiation damaged cells of Tradescantia and this, they concluded, is



a

10μm



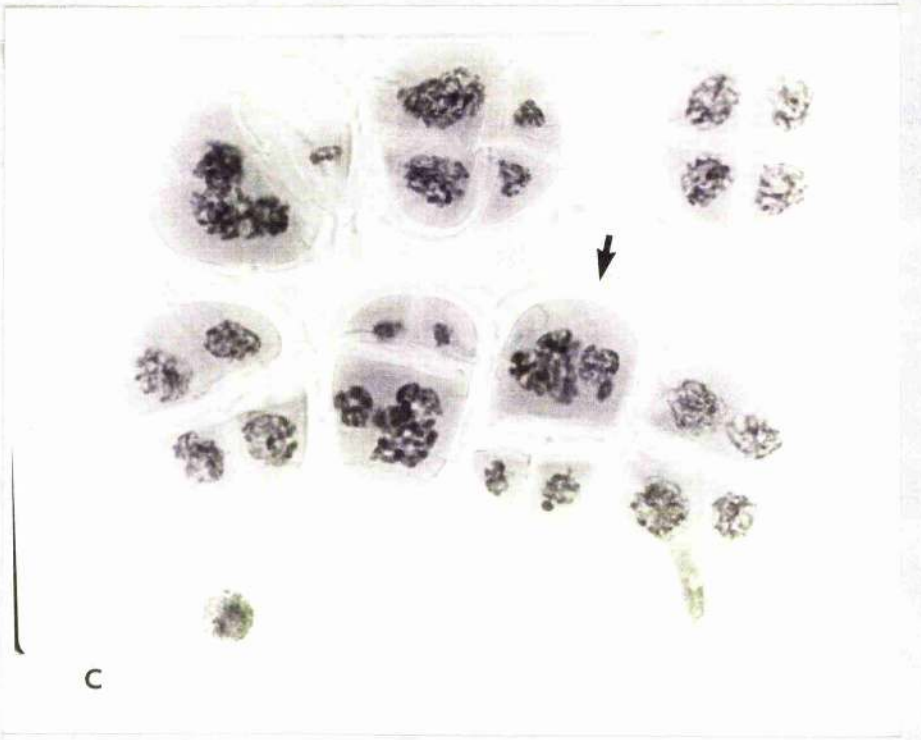
b

10μm

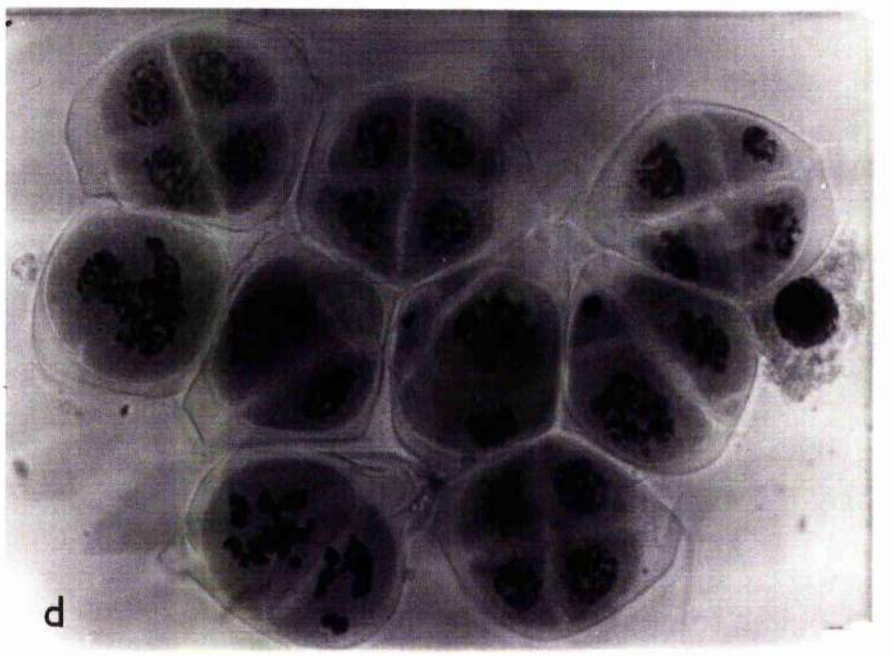
PLATE IV.8. Abnormality in Cell Division

a - Figure shows non-synchronisation of the two daughter cells after A-I.

b - The two dyad-cells showing considerably variable number of chromosomes.



40µm



40µm

PLATE IV.8. Abnormality in Cell Division (contd.)

c & d - Tetrad stage of meiosis in the abnormal plant. Note delayed cytokinesis in the daughter cell having the higher number of chromosomes(arrow).

due to the genetic differences between the daughter nuclei formed at interphase. It was also evident that the chromosome separation at anaphase-II, like that of the first division, was incomplete in some cases, once again leading to unequal separation.

The abnormality seems to be very similar to that of Allium ascalonicum reported by Darlington & Haque (1955). These authors demonstrated that a particular zone in the anther lobe was affected and the plant was male sterile. Male-sterility in rye is of less significance because of its allogamous breeding system, but this does not exclude the possibility that such genotypes may occur. However, the plant had a reasonably good seed-set (52%) in spite of its poor growth (height 78 cms with two tillers). This indicates that the plant was normally fertile on the female side and the abnormalities observed in the PMCs are probably a manifestation of its male sterile characteristics.

GENERAL DISCUSSION

It has been pointed out earlier that there is a great deal of controversy as to the relationship between chromosome pairing pattern and seed-set in autotetraploid rye. One group of investigations demonstrates that an increasing frequency of quadrivalents associated with increased chiasma frequency leads to an improvement in fertility (Roseweir & Rees, 1962; Hazarika & Rees, 1967) while another set of studies suggests that higher bivalent frequency is associated with improved fertility (Plarre, 1954; Bremer & Bremer-Reinders, 1954; Hilpert, 1957; Aastveit, 1968).

In rye where quadrivalents disjoin in a two-by-two manner giving equal chromosomal separation (Roseweir & Rees, l.c.; Hazarika & Rees, l.c.), as with bivalents, both quadrivalents and bivalents seem to be realistic alternatives for balanced gamete formations insofar as chiasma frequency remains unaltered. The choice between these two configurations should, therefore, be made considering which of the two configurations would ensure a better meiotic stability and thus a stability in seed-set. For convenience, the discussion is separated into two parts, the first part deals with the problem of chromosome pairing behaviour in view of meiotic stability and the second part is devoted to the problem of fertility, although the latter is not independent of the former.

Chromosome associations in autotetraploids

It has been well demonstrated that higher chiasma frequency is a pre-requisite for higher quadrivalent frequency (Roseweir & Rees, 1962; Nazarika & Rees, 1967; Crowley & Rees, 1968; Swami & Thomas, 1968). Whether an increase in bivalent frequency in a tetraploid can also accompany high chiasma frequency has not been as well documented as in the case of quadrivalents. In fact in inbred materials (Roseweir & Rees, l.c.; Nazarika & Rees, l.c.) and in autogamous species (Swami & Thomas, l.c.) bivalent frequency has been shown to be negatively correlated with chiasma frequency. But the results obtained in the present study clearly indicate that bivalent frequency can increase without a decrease in chiasma frequency. This was observed in two ways. First, the mean chiasma frequency of the three populations were similar (no significant difference) while the average frequency of quadrivalents and bivalents differed significantly between populations. Second, the populations which had less or no inbreeding effect showed a positive correlation between chiasma frequency and bivalent frequency.

Similar results have been reported for other autotetraploids. Thus Swaminathan & Sulbha (1959) observed a decrease in the number of quadrivalents in autotetraploid Brassica campestris in the selected material, although chiasma frequency remained unaltered. Results obtained for tetraploid maize (Giles & Randolph, 1951) and tetraploid barley (Bender & Gaul, 1966)

also seem to suggest that bivalent frequency can be increased without a necessary reduction in chiasma frequency.

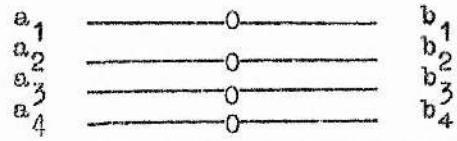
This kind of change in the frequencies of different types of configurations has been suggested to be due to the re-distribution of chiasmata (Crowley & Rees, 1968). The latter may increase quadrivalent frequency as demonstrated in Lolium (Crowley & Rees, l.c.) or it may increase bivalent frequency as mentioned above. The basic important point is that a change in chromosome association pattern is possible without a change in chiasma frequency.

Now we can consider whether quadrivalents or bivalents offer a better prospect of meiotic stability. The results obtained from the experiments on the effects of environmental factors on chromosome associations (Section Four) suggest that (i) bivalent formations are more easily and efficiently accomplished than multivalent formations, (ii) bivalents are less sensitive to environmental fluctuations than multivalents and (iii) bivalents guarantee equal chromosomal separation at anaphase-I while some quadrivalents may fail to do so. These facts lead to the conclusion that bivalents will ensure the meiotic stability better than the multivalents. Furthermore, Timmis & Rees (1971) showed that in rye there already exists a (natural) preference for bivalent formations as opposed to multivalents. Therefore, any effort to increase the frequency of quadrivalents to 100% is bound to be a failure. This will

never be achieved even in a strict autotetraploid. Therefore, the only alternative is to make efforts to increase the frequency of bivalents. Several attempts have already been made to induce preferential pairing in autotetraploids but with no distinct success so far. However, there seems to be some prospect in this direction and this will be taken up later. In the meantime it is worthwhile to examine the basic principle involved in the chromosome association pattern in autotetraploids to discuss the prospects of manipulation of the pairing system.

In a true autotetraploid the affinity between the four homologous chromosomes is equal. With a single point of pairing initiation along the entire chromosome length only bivalents will be formed, because partner exchange is excluded. With two localised points of pairing initiation, the four homologous chromosomes can form either two bivalents or a single quadrivalent. Theoretically the ratio between bivalent pairs and quadrivalents will be 1:2 on the condition that the two points are far enough from each other to avoid interference (Sybenga, 1972). This is illustrated in the table below. It will be seen from the table that 1/3 of the chromosomes are involved in bivalent formations while the remainder 2/3 in quadrivalent formations.

Table A. Metaphase-I configurations in an autotetraploid where pairing starts at the two ends of the chromosome.

FOUR HOMOLOGUES:			
Chromosome associations at end a	Chromosome associations at end b		
	$b_1 - b_2$ $b_3 - b_4$	$b_1 - b_3$ $b_2 - b_4$	$b_1 - b_4$ $b_2 - b_3$
$a_1 - a_2$ $a_3 - a_4$	II + II	IV	IV
$a_1 - a_3$ $a_2 - a_4$	IV	II + II	IV
$a_1 - a_4$ $a_2 - a_3$	IV	IV	II + II
TOTAL	3 (II+II) ; 6 IV		

If pairing is initiated at any additional points the chances of partner exchange will increase correspondingly, with a consequence of higher frequency of multivalents and fewer bivalents. The factors that may limit partner exchanges are (i) rigidity of the chromosomes, (ii) rapid progress of pairing from the initiation point along the chromosome length and (ii) several initiation points close together may attract the same partner.

In addition localisation of chiasmata may also affect the

proportion of multivalents. For example, if chiasmata are localised only at one side of the exchange point, the configurations at metaphase-I will all be bivalents, in spite of multivalent association at zygotene-pachytene (Sybenga, 1972). This is apparently the case in tetraploid teosinte, Zea mexicana (Shaver, 1962) and tetraploid Vaccinium australe (Jelencovic & Harrington, 1971).

In normal genotypes of rye, the pairing initiation is restricted to the two ends of the chromosome. According to the model illustrated in the table above 1/3 of the chromosomes would be involved in bivalent and 2/3 in quadrivalent formations. Deviations from this would indicate a change from the two points system, which may be due to genetic or structural differentiation of the chromosomes. Sybenga (1972) reports that approximately 2/3 of the rye chromosomes are in fact involved in quadrivalent formations which supports the hypothesis of two points pairing initiation in rye.

The three rye populations investigated here showed significant deviations from the expected 2/3 chromosomes in quadrivalents and 1/3 in bivalent formations (table B below). In each population there was a preponderance of the number of chromosomes involved in bivalents and a deficiency in the number of chromosomes in quadrivalent formations, the deviation being the greatest in the "high" population (see table B).

Table B. Chi-square test* for the number of chromosomes involved in quadrivalent and bivalent formations according to two points pairing initiation at the two ends of the chromosomes in different rye populations.

Population	Chi-Square	D.F.	Probability
High	13.832	1	0.001
Low	8.409	1	0.001 - 0.01
Unselected	6.879	1	0.001 - 0.01
Inbred Lines (Hazarika & Rees, 1967)	2.102	1	0.10 - 0.20

* The number of chromosomes involved in trivalents and univalents were deducted from the total number of chromosomes (=28) and chi-square was computed from the remainder in each population.

These results demonstrate, first, that in each of the three populations investigated, there is a restriction upon multivalent formation as Timmis & Rees (1971) found and, second, a response of selection in favour of bivalent formations in the "high" population. In other words, in these populations, of the two points of pairing initiation one takes part in partner exchange more effectively than the other, thus reducing the number of multivalents. And this happens more frequently in the "high" population.

In contrast, the insignificant chi-square for the inbred lines (bottom line in table B above) suggests that pairing initiations at both ends of the chromosome are equally effective,

thus giving no significant deviation from the expected proportion of the number of chromosomes in quadrivalent and bivalent formations. This is apparently because, as suggested earlier, inbreeding helps maintain homology of the chromosomes and restricts chromosomal differentiation whether it is genetic or structural.

This brings back the question, how does the pairing differentiation take place? The mechanism is not well understood. However, Sybenga (1969) summarised the various hypotheses put forward to explain the natural differentiating mechanism. These are relevant here and are as follows:

1. The first of the several stages of chromosome association, the long-distance attraction is decisive for final association of homologues or homeologous chromosomes by chiasmata. This attraction is localised in a limited number of sites (zygomeres) that are specific in action (i.e. a zygomere of one type does not attract one of another type). Thus a differentiation may take place by a change in the zygomere activity or originally identical zygomeres may mutate to show differential specificity (Sybenga, 1969).

2. Different timing patterns of chromosomal processes such as attraction, condensation and DNA synthesis may suppress meiotic association (Endrizzi, 1962).

3. The well known effect of $5B^{L}$ chromosome of wheat (Riley & Chapman, 1958; Sears & Okamato, 1958) resulting in the suppression of homeologous associations may act through a modification in the somatic pairing in the pre-meiotic mitosis by bringing the homologous chromosomes in close vicinity (Feldman, 1966).

4. Structural differences (deficiency, duplications, inversions, translocations) may induce preferential pairing.

Of these, the role of structural differences in pairing differentiation has often been emphasised, probably because chromosomal rearrangements are easily made available and are relatively easily studied (Stebbins, 1956; Mac Key, 1956; Gaul, 1958; Doyle, 1963; Shaver, 1963; Sybenga, 1969 & 1973). Grell (1963) demonstrated that an inversion in triploid Drosophila reduced pairing between the inverted and the normal chromosomes and with seven inversions the pairing differentiation was complete. Shaver (1963) observed that a tetraploid maize, heterozygous for an inversion present in duplex condition, showed preferential pairing for the two inverted chromosomes and the two normal chromosomes. In a/maize-teosinte hybrid the same inversion was highly effective in inducing preferential pairing. Whereas in other organisms, for instance in tetraploid Rhoeo discolor no indication for preferential pairing was observed, although each chromosome in the genome was involved in a translocation (Walters & Gerstel, 1948).

In rye, examples of both kinds are available. Ahloowalia (1963) found no evidence of preferential pairing in a tetraploid genotype in which a/translocation was present in a duplex condition. On the other hand, Sybenga (1973) reported that while some of the translocations induced a degree of preferential pairing, others failed to do so (see also Sybenga, 1965, 1966a, 1972a, b).

This led him to conclude that the conversion of a free pairing system of the four homologues of an autotetraploid to the restricted two-by-two pairing pattern requires a combination of structural rearrangement and naturally occurring differences (presumably genetic) in the chromosome attraction system.

The role of structural differences alone in differentiating pairing pattern seems, therefore, very limited. This becomes more apparent if one examines the situation in species or species-hybrids where natural differentiation has occurred. Perhaps the most suitable example is Primula kewensis (Upcott, 1939), where pairing in the doubled hybrid is largely confined to chromosomes derived from the same parent, although homœologous pairing takes place in the undoubled hybrid. This kind of preferential pairing, not unjustifiably, was attributed to structural differences. Ironically there are many instances in which no clear structural differences have been found in spite of preferential pairing. Subsequently the emphasis was shifted to cryptic structural differences. Later with the discovery of genetic control of pairing pattern, as in wheat (Riley & Chapman, 1958) and few others, for instance tetraploid Poa annua (Ellis, et al, 1973), the unequivocal role of cryptic structural differences comes under question and in many cases seems inadequate, although this might have played a part in conjunction with genetic differentiation.

Our "high" population show some effects of selection in

that there is a significant reduction in quadrivalent frequency with a corresponding increase in bivalent frequency. This is probably due to genetic differentiation, rather than structural. It is, however, doubtful whether this difference will be maintained once the selection pressure is released. There are two reasons why it may not be so. First, in some plants quadrivalent frequency is higher than in others, some PMCs even showed seven quadrivalents, which suggests that the differentiation is not uniform throughout the selected population. With the release of selection pressure the frequency of those individuals in which differentiation is more pronounced will be lowered because of random mating involving plants without or with less differentiation. Second, if within a particular plant the differentiation is incomplete, for instance, three normal chromosomes against one differentiated, as opposed to two-by-two differentiation, the later generations will increase the frequency of normal undifferentiated chromosomes and the population will revert to high frequency of multivalents. For these reasons it has been often suggested that chromosomal differentiation should be introduced prior to chromosome doubling.

The latter approach seems to be more promising if genetically different ecotypes or lines with chromosomal rearrangements are crossed at the diploid level followed by chromosome doubling as demonstrated by Stebbins (1956) in Dactylis. This may prove fruitful in rye as well, as shown in tetraploid hybrid of Secale cereale x S. montanum where several

reciprocal translocations were highly effective in inducing disomic pairing. In short, the pre-conditions can be laid down as follows:

(1) Two diploid ecotypes or lines should be genetically different.

(2) Chromosome rearrangements in the form of translocation, inversion, duplication, etc. should be introduced at the diploid level.

(3) Rearrangements should be made within the pairing initiation region. With the initiation region intact the rearrangements would not show differential specificity in chromosome pairing (Sybenga, 1969).

(4) Crossing should be made between the ecotypes or lines followed by chromosome doubling. This in turn should be followed by further selection.

One precaution should be made here that meiotic balance may not be obtained immediately after chromosome doubling. In that case, it would be necessary to pursue a selection programme which may eventually evolve a regular and reliable mechanism of chromosome behaviour. The selection will also re-inforce one or more of the following underlying causes of preferential pairing.

(a) Differential affinity between chromosomes due to zygomere changes (Sybenga, 1966c).

(b) Differentiation in coiling pattern (Endrizzi, 1962).

(c) Genetical control mechanism as in wheat.

In view of the prospects of the manipulation of chromosome pairing system in tetraploid rye, Tarkowski's (1970) observation is encouraging. He seems to have observed chromosomal differentiation in "pairs" with respect to chromosome condensation, arrangement of chromosome arms and gradual disappearance of secondary constriction in varieties of tetraploid rye. Otlowaska (1971) claims to have increased bivalent frequency to 12.15 and 12.31 per PMC in two varieties of tetraploid rye, one obtained by backcrossing hexaploid Triticale to rye and the other derived from crossing of different tetraploid ecotypes. It, therefore, seems that further work along the line outlined above may be worthwhile undertaking.

Fertility in autotetraploids

Several autotetraploid species have been established and proved successful in nature at a relatively low level of meiotic regularity (e.g. Dactylis glomerata, Solanum tuberosum, Hordeum bulbusum), comparable to that of tetraploid rye populations. These natural species are at an advantage because of their vegetative mode of reproduction or perenniality or both, so that fertility has not been as important a factor as in the case of rye or other annual grain crops. It is because of the latter that the meiotic behaviour needs to be improved in rye in order to exploit the full advantage of chromosome doubling.

So far the fertility problem in tetraploid rye has received

one sided attention, i.e. from the cytogenetic point of view. Although it is not difficult to see why it has been so, other factors, genic or physiological, are not less important. We have already demonstrated that factors of physiological nature also play an important part in determining fertility and it was shown that these factors act independently of the cytological factors. This is more so in populations which have recovered from the initial genetic imbalance of chromosome doubling and have reached a threshold state of stability (cf. the unselected population).

From the cytological viewpoint it has been well established that chiasma frequency, being genotypically controlled, influences the pairing configurations that facilitate balanced gamete formation (Nazarika & Rees, 1967). Thus an increase in chiasma frequency improves the fertility. But in populations where the chiasma frequency has already reached the optimum level, a further increase in fertility will depend on meiotic stability so that there are always quadrivalent and/or bivalent formations and no trivalent or univalent formation. For reasons discussed above 100% quadrivalent formation can never be guaranteed in an autotetraploid. On the other hand, obligatory pairing leading to all bivalent configurations seems to be equally improbable at present. However, examples of autotetraploids where pairing differentiation has occurred (e.g. Phleum, Teosinte) or where differentiation has progressed to a large extent (e.g. Lotus corniculatus, Medicago sativa,

Anthoxanthum odoratum) provide encouragements for experimental manoeuvres. The possible means of doing so has been discussed and outlined above.

It appears that in most of the advanced populations of autotetraploids, where chiasma frequency has reached the optimum level, an increase in bivalent frequency is more favourable for fertility than quadrivalents. This is supported by Aastveit's (1968) observation in several strains of tetraploid rye. He found that the strains with higher bivalent frequency had the higher seed-set. Similarly the results obtained by Swaminathan & Sulbha (1959) in autotetraploid *Brassica campestris* after 19 generations of selection for high fertility showed a decrease in the number of quadrivalents, although chiasma frequency remained unaltered. Similar results have been reported for maize (Giles & Randolph, 1951) and barley (Bender & Gaul, 1966). In the present investigation we have not been able to demonstrate an increase in seed-set in the "high" population as compared to the unselected population, although in the former bivalent frequency was increased and quadrivalent frequency reduced. This was mainly due to the inbreeding depression in the "high" population. In spite of this there was no significant difference in seed-set between the two populations. If inbreeding depression is taken into account, the "high" population would appear to have performed rather well, possibly better than the unselected population. It thus appears that except in inbred materials (Roseweir & Rees, 1962;

Hazarika & Rees, 1967), bivalents are favourable configurations for fertility. The reason is, chiasma frequency is at a low level in inbred materials, as a result the partner exchange is reduced leading to a high frequency of unpaired chromosomes. The consequence is a reduction in seed-set. However, whenever the partner exchange is possible with adequate chiasmata being available in inbred materials, the tendency is to form a quadrivalent. This is presumably because the pairing initiation points or zygomeres are identical in inbred lines and, therefore, have greater affinity to attract one another. In other words, the normal pairing pattern in inbred lines is polysomic while in outbred materials the pairing pattern may vary from polysomic to disomic, depending on the affinity among the homologous chromosomes. This was supported by the higher frequency of multivalents in the inbred lines as compared to that in the unselected population, in spite of the reduced chiasma frequency in the former (see Section One, table I.10).

The other factor which is important in determining fertility is the frequency of aneuploids. The spores and zygotes in these individuals have a reduced probability of normal development and thus affect the fertility. This is often influenced by the background genotype and environmental conditions. Ellerström & Sjodin (1963) showed that with higher nutrients supplied to the plant, the seed-set was increased. The cause in this case was not the absence of aneuploids in the

population, but a better development of aneuploid zygotes. Moore (1963) reported that the aneuploids which survived at Davies (California) were much more vigorous than their counterparts at Svalöf (Sweden). This was again due to the better opportunities for the aneuploids for development in a more favourable climate at Davies. Moore's results also suggest that the fertility of both euploids and aneuploids was higher at Davies than at Svalöf. In the present investigation, the "high" population grown in 1970, having received an additional fertiliser treatment, showed a significant increase in seed-set over the "low" population but the difference was not realised in the following year (1971) when the two populations were grown under the same conditions. Ising (1967) working with autotetraploid barley found a better fertility in F_1 plants with strong hybrid vigour than those with less or no hybrid vigour. Once again the cause was a better development of aneuploids. In such cases the apparent increase in fertility will be compensated for in later generations because of increased frequency of aneuploids which would depress the average of the population.

Apart from aneuploidy, genic imbalance can often lead to abnormal development or death of a certain percentage of gametes, zygotes or endosperms. In the present investigation, we found an instance of abnormal spindle activity and cell-wall formation resulting in highly imbalanced gametes which apparently died. Rosemark (1967) reported an instance

of abnormal cell wall development in tetrads of autotetraploid sugarbeet which resulted in constrictions and fragmentations of a large number of nuclei. Such extreme deviations are, of course, rare in natural populations, but disturbances in the normal process of meiotic or mitotic divisions, such as anaphase-I bridges and fragments due to chromosome breakage and re-union, polyad formation, translocation, inversions, etc. seem to be relatively common. Selection will gradually remove such abnormalities.

Furthermore, factors of physiological nature are also important in fertility. As shown already, such factors can mask the correlation of meiotic features with fertility and thus demonstrates their independence from cytological factors. Therefore, selection for physiological factors seems to be as important as the cytological factors for the improvement of fertility.

In conclusion, selection is important not only for fertility but also for adjustment of all characters to the tetraploid level. When such adjustments have been achieved, only then will the full advantage of chromosome doubling be realised.

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A P P E N D I X

Appendix Table I.A. Meiotic Features and Seed-Set in Haploid Plants of High Population, 1972.

No. of PMCs	Chiasma Frequency	Cell-variance for chiasmata	IV Frequency	III Frequency	II Frequency	I Frequency	No. of Chromosomes in IV+II	No. of Chromosomes in III+I	D.I. Ang. value	Regular Tetrads Ang. value	Seed-set Ang. value
20	24.85	1.397	1.90	0.10	9.90	0.30	27.40	0.60	63.43	67.13	64.16
20	23.95	2.155	1.80	0.40	9.35	0.90	25.90	2.10	39.23	64.67	58.05
20	24.20	3.379	2.25	0.35	8.80	0.35	26.60	1.40	53.73	67.21	59.34
20	25.30	3.063	2.75	0.05	8.35	0.15	27.70	0.30	71.57	72.74	51.35
20	25.65	2.345	1.65	0.05	10.45	0.35	27.50	0.50	67.21	72.95	60.00
20	25.60	2.358	2.30	0.05	9.25	0.15	27.70	0.30	71.57	68.78	58.69
20	24.75	2.197	2.15	0.25	9.20	0.25	27.00	1.00	63.43	68.03	71.57
20	25.95	2.261	2.70	0.15	8.25	0.25	27.30	0.70	63.43	71.43	61.34
14	19.50	5.962	1.86	1.28	7.36	2.00	22.16	5.84	28.57	50.53	29.33
20	23.00	5.790	1.90	0.35	9.30	0.75	26.20	1.80	45.00	60.53	45.00
20	25.95	1.734	1.95	0.15	9.75	0.25	27.30	0.70	63.43	74.00	74.66
20	24.25	3.461	2.00	0.30	9.15	0.80	26.30	1.70	47.87	67.54	43.85
20	22.60	2.463	2.05	0.60	8.60	0.80	25.40	2.60	42.13	61.82	56.17
18	24.00	4.235	2.06	0.27	9.11	0.13	26.46	0.94	61.89	66.58	68.03
20	24.20	3.958	2.35	0.05	8.80	0.85	27.00	1.00	56.79	65.20	53.73
20	24.40	2.674	2.10	0.25	9.20	0.45	26.80	1.20	56.79	65.96	61.34
18	23.56	2.850	1.45	0.33	10.33	0.55	11.78	0.88	50.77	73.05	66.42
20	25.25	2.958	2.50	0.15	8.65	0.25	27.30	0.70	63.43	69.12	63.43
20	23.45	3.208	1.85	0.35	9.45	0.65	26.30	1.70	53.73	57.23	57.42
20	25.85	1.397	1.75	0.10	10.25	0.20	27.50	0.50	67.21	69.56	48.45
20	26.60	1.200	1.75	0.05	10.40	0.05	27.80	0.20	77.08	73.57	58.05
20	24.20	3.853	2.20	0.10	9.15	0.60	27.10	0.90	56.79	65.57	50.18
20	24.25	2.197	1.95	0.35	9.35	0.45	26.50	1.50	53.73	67.05	39.82
20	23.95	3.103	2.05	0.10	9.40	0.70	27.00	1.00	53.73	64.97	31.95
20	24.60	3.516	3.00	0.15	7.55	0.45	27.10	0.90	60.00	68.28	63.43
20	25.95	1.313	2.70	0.10	8.40	0.10	27.60	0.40	71.57	75.00	56.17
20	24.90	2.621	2.00	0.25	9.45	0.35	26.90	1.10	56.79	69.56	62.03
20	25.80	1.432	3.15	0.10	7.40	0.30	27.40	0.60	63.43	73.05	60.00
20	25.20	2.379	2.55	0.00	8.80	0.20	27.80	0.20	71.57	71.47	71.57
20	25.65	1.292	1.95	0.20	9.70	0.20	27.20	0.80	63.43	70.27	55.55
20	24.35	3.713	2.30	0.25	8.70	0.65	26.60	1.40	50.77	63.72	56.17
20	25.25	1.987	2.05	0.15	9.50	0.35	27.20	0.80	60.00	69.30	51.94
20	23.25	4.513	1.95	0.45	9.05	0.75	25.90	2.10	47.87	67.62	50.18
20	23.95	2.682	2.20	0.30	8.90	0.50	26.60	1.40	53.73	64.09	56.79
20	24.35	2.555	1.95	0.10	9.75	0.40	27.30	0.70	63.43	71.19	62.73
20	24.25	4.513	2.50	0.20	8.40	0.60	26.80	1.20	53.73	63.15	56.17
20	26.65	1.292	2.40	0.00	9.20	0.00	28.00	0.00	88.19	77.75	60.67
20	26.00	2.526	3.25	0.10	7.30	0.10	27.60	0.40	77.08	72.15	48.45
20	24.65	2.450	2.25	0.05	9.20	0.45	27.40	0.60	60.00	69.04	51.94
20	25.35	2.240	2.45	0.05	8.95	0.15	27.70	0.30	71.57	72.05	60.67

Appendix Table I.B. Meiotic Features and Seed-Set in Euploid Plants of Low Populations, 1972.

No. of PMCs	Chiasma Frequency	Cell variance for chiasmata	IV Frequency	III Frequency	II Frequency	I Frequency	No. of Chromosomes in IV+II	No. of Chromosomes in III+I	D.I. Ang. value	Regular Tetrads Ang. value	Seed-set Ang. value
20	26.00	1.789	2.50	0.15	8.70	0.15	27.40	0.60	67.21	75.00	40.40
20	24.75	3.776	2.60	0.20	8.30	0.40	27.00	1.00	63.43	68.95	68.03
20	26.10	1.779	2.80	0.00	8.40	0.00	28.00	0.00	88.19	70.63	25.85
20	25.90	2.200	2.15	0.05	9.55	0.15	27.70	0.30	71.57	71.09	55.55
18	25.06	1.938	2.56	0.05	8.72	0.17	27.68	0.32	70.54	72.64	67.21
20	25.30	1.905	2.80	0.10	8.10	0.30	27.40	0.60	67.21	68.87	19.37
20	24.50	1.737	2.40	0.15	8.85	0.25	27.30	0.70	63.43	66.82	42.71
20	24.85	1.713	2.50	0.10	8.70	0.30	27.40	0.60	63.43	65.27	45.00
20	25.60	2.253	2.40	0.15	8.85	0.25	27.30	0.70	63.43	65.50	57.42
20	24.75	3.461	2.85	0.35	7.40	0.75	26.20	1.80	50.77	61.14	60.00
20	25.05	1.734	2.65	0.30	8.00	0.50	26.60	1.40	53.73	60.87	55.55
20	25.05	2.787	2.80	0.45	7.50	0.45	26.20	1.80	50.77	63.43	51.35
20	25.25	1.461	2.15	0.15	9.35	0.25	27.30	0.70	63.43	66.03	63.43
20	24.95	3.524	2.75	0.35	7.70	0.55	26.40	1.60	60.00	65.88	62.73
20	24.75	3.145	2.50	0.25	8.45	0.35	26.90	1.10	70.00	66.03	50.18
20	25.30	2.537	3.30	0.20	7.00	0.20	27.20	0.80	63.43	68.61	63.43
20	25.05	2.155	2.80	0.20	7.95	0.30	27.10	0.90	60.00	63.01	58.69
20	25.60	2.358	3.10	0.20	7.35	0.30	27.10	0.90	60.00	67.54	55.55
20	24.70	1.589	3.25	0.20	7.05	0.30	27.10	0.90	60.00	66.66	53.73
20	25.00	2.211	2.80	0.35	7.65	0.45	26.50	1.50	56.79	68.03	54.94
20	24.45	2.682	2.90	0.35	7.35	0.65	26.30	1.70	45.00	63.94	60.67
20	24.65	1.503	3.60	0.15	6.35	0.45	27.10	0.90	60.00	69.30	51.94
20	23.75	2.724	2.50	0.45	7.90	0.85	25.80	2.20	42.13	64.82	49.60

Appendix Table I.C. Meiotic Features and Seed-Set in Euploid Plants of Unselected Populations, 1972.

No. of PMCs	Chiasma Frequency	Cell variance for chiasmata	IV Frequency	III Frequency	II Frequency	I Frequency	No. of Chromosomes in IV+II	No. of Chromosomes in III+I	D.I. Ang. value	Regular Tetrads Ang. value	Seed-set Ang. value
20	25.40	2.042	2.65	0.35	7.90	0.55	26.40	0.60	47.87	58.56	63.03
20	25.05	4.682	2.85	0.20	7.80	0.40	27.00	1.00	56.79	66.67	62.03
20	24.60	2.568	2.95	0.40	7.15	0.70	26.10	1.90	45.00	65.12	49.60
20	25.75	2.408	2.80	0.20	8.00	0.20	27.20	0.80	63.43	64.09	43.85
20	25.65	2.555	2.25	0.30	8.75	0.60	26.50	1.50	53.73	67.46	56.79
20	25.75	2.303	2.50	0.05	8.90	0.05	27.80	0.20	77.08	72.05	60.00
20	25.20	1.958	2.80	0.25	7.80	0.45	26.80	1.20	53.73	66.89	58.05
20	25.75	1.105	3.20	0.50	6.60	0.50	26.00	2.00	47.87	71.57	67.21
20	24.25	4.408	2.55	0.20	8.30	0.60	26.80	1.20	53.73	68.61	56.17
20	25.50	2.895	2.20	0.15	9.10	0.55	27.00	1.00	60.00	67.13	56.79
20	25.65	2.450	2.90	0.15	7.80	0.35	27.80	0.20	60.00	69.12	63.43
20	25.80	1.432	2.30	0.20	8.90	0.40	27.00	1.00	60.00	69.47	62.03
20	25.05	3.945	2.70	0.15	8.25	0.25	27.30	0.70	67.21	67.70	60.67
20	24.15	2.450	2.50	0.30	8.25	0.60	26.50	1.50	50.77	62.73	64.16
20	22.30	2.642	2.50	0.65	7.40	1.25	24.80	3.20	36.27	49.31	60.67
20	25.20	1.116	2.60	0.25	8.30	0.25	27.00	1.00	60.00	70.27	61.34
20	25.65	1.818	2.90	0.10	8.00	0.10	27.60	0.40	71.57	66.82	68.03
20	25.95	1.418	2.40	0.30	8.60	0.30	26.80	1.20	56.79	66.27	60.00
20	25.75	1.776	2.95	0.10	7.90	0.10	27.60	0.40	71.57	79.37	51.35
20	24.20	2.379	2.20	0.35	8.80	0.55	26.40	1.60	47.87	65.65	58.05
20	24.75	3.250	2.75	0.15	8.20	0.15	27.40	0.60	67.21	65.05	58.05
20	25.40	2.147	2.90	0.20	7.80	0.20	27.20	0.80	63.43	66.11	54.33
20	24.40	1.937	2.85	0.25	7.70	0.45	26.80	1.20	53.73	63.58	50.77
20	23.35	2.450	3.05	0.30	7.05	0.80	26.30	1.70	47.87	57.99	51.35
20	24.50	1.947	2.50	0.25	8.50	0.25	27.00	1.00	60.00	64.60	56.79
20	25.45	4.366	2.50	0.20	8.55	0.30	27.10	0.90	60.00	67.29	67.21
20	24.25	2.618	2.55	0.20	8.35	0.50	26.90	1.10	56.79	68.36	50.77
20	25.35	2.239	2.85	0.25	7.80	0.25	27.00	1.00	60.00	59.60	63.43
20	25.40	1.516	3.10	0.15	7.50	0.15	27.40	0.60	67.21	69.04	50.77
20	25.05	1.734	2.70	0.15	8.15	0.45	27.10	0.90	56.79	63.79	63.43
20	23.35	1.924	2.65	0.45	7.55	0.95	25.70	2.30	42.13	54.94	43.85
20	25.60	2.358	2.60	0.20	8.40	0.20	27.20	0.80	63.43	63.65	56.79
20	24.50	2.895	2.90	0.10	7.95	0.20	27.50	0.50	67.21	60.94	62.73
20	25.25	1.671	2.80	0.20	7.95	0.30	27.10	0.90	60.00	64.82	55.55
20	24.75	3.566	2.55	0.20	8.50	0.20	27.20	0.80	63.43	66.89	54.33
20	25.80	1.116	2.85	0.10	8.10	0.10	27.60	0.40	71.57	62.82	55.55
20	25.15	3.082	3.10	0.30	7.20	0.30	26.80	1.20	56.79	63.87	62.03
20	25.40	2.147	2.65	0.25	8.10	0.45	26.80	1.20	56.79	70.00	57.42
20	25.30	1.695	2.40	0.25	8.65	0.35	26.90	1.10	60.00	64.90	64.16
20	25.85	1.292	2.35	0.15	8.90	0.35	27.20	0.80	60.00	68.03	62.73

Appendix Table 2.A. Morphological Characters and Seed-Set in Euploid Plants of High Populations, 1972.

Plant No.	Plant Height (cms)	No. of Tillers	No. of Spikelets per Spike	Spike length (cms)	Seed-set (Ang. value)
1	136	6	31	11.50	64.16
2	132	8	27	11.00	58.05
3	133	5	19	8.00	59.34
4	126	4	31	12.40	51.35
5	99	7	32	12.20	60.00
6	133	5	33	11.40	58.69
7	130	5	24	10.50	71.57
8	148	8	24	11.00	61.34
9	121	5	21	9.00	29.33
10	117	8	24	9.00	45.00
11	116	5	22	8.00	74.66
12	113	5	27	12.50	43.85
13	143	8	34	12.00	56.17
14	131	6	28	12.50	68.03
15	112	2	24	9.50	53.73
16	118	5	26	11.30	61.34
17	138	4	26	11.40	66.42
18	128	6	28	11.60	63.43
19	126	4	24	10.00	57.42
20	119	7	24	8.60	48.45
21	123	2	23	8.00	58.05
22	93	8	27	10.50	50.18
23	96	4	22	8.70	39.82
24	107	5	14	6.50	31.95
25	118	3	25	10.80	63.43
26	146	9	24	12.00	56.17
27	142	5	30	14.00	62.03
28	121	3	22	10.00	60.00
29	113	6	30	11.50	71.57
30	136	4	22	9.00	55.55
31	146	5	24	10.50	56.17
32	173	10	32	12.10	51.94
33	131	7	28	12.10	50.18
34	125	7	28	11.80	56.79
35	122	10	31	10.50	62.73
36	143	5	27	9.40	56.17
37	145	5	33	12.10	60.67
38	118	7	27	11.80	48.45
39	156	8	26	12.40	51.54
40	130	3	34	11.60	60.67

Appendix Table 2.B. Morphological Characters and Seed-Set in Euploid Plants of Low Populations, 1972.

Plant No.	Plant Height (cms)	No. of Tillers	No. of Spikelets per Spike	Spike length (cms)	Seed-set (Ang. value)
1	127	5	18	9.50	40.40
2	116	4	25	11.00	68.03
3	149	4	24	13.00	25.85
4	116	9	25	10.20	55.55
5	139	7	23	8.50	67.21
6	117	4	28	12.00	19.37
7	114	7	26	11.70	42.71
8	100	9	25	9.90	45.00
9	126	4	24	10.00	57.42
10	156	9	31	13.00	60.00
11	133	5	30	10.50	63.43
12	155	6	30	12.00	55.55
13	140	5	31	11.00	51.35
14	149	7	30	12.00	62.73
15	140	8	21	11.50	50.18
16	155	5	25	11.00	63.43
17	123	6	30	12.00	58.69
18	149	17	30	13.00	55.55
19	139	12	30	12.30	53.73
20	141	9	23	11.50	54.94
21	138	5	25	10.90	60.67
22	117	8	30	12.30	51.94
23	141	18	32	12.50	49.60

Appendix Table 2.C. Morphological Characters and Seed-Set in Euploid Plants of Unselected Populations, 1972.

Plant No.	Plant Height (cms)	No. of Tillers	No. of Spikelets per Spike	Spike length (cms)	Seed-set (Ang. value)
1	147	6	29	11.70	63.03
2	126	3	20	12.20	62.03
3	129	5	31	13.70	49.60
4	133	8	26	10.70	43.85
5	131	3	29	10.80	56.79
6	141	14	22	10.90	61.34
7	154	6	25	10.70	58.05
8	134	6	26	11.90	67.21
9	152	7	31	14.20	56.17
10	146	1	25	11.80	56.79
11	154	6	27	10.50	63.43
12	141	7	27	10.70	62.03
13	131	9	25	11.50	60.67
14	160	11	26	10.10	64.16
15	164	8	31	13.60	60.67
16	138	7	31	12.70	61.34
17	147	4	29	12.10	68.03
18	141	6	30	12.30	60.00
19	148	10	31	14.00	51.35
20	149	11	32	14.40	58.05
21	141	7	30	11.70	58.05
22	146	10	31	12.50	54.33
23	151	6	24	10.00	50.77
24	149	13	31	12.00	51.35
25	121	8	27	10.90	56.79
26	150	7	33	13.20	67.21
27	142	8	26	14.60	50.77
28	144	16	27	12.30	63.43
29	142	8	26	10.90	50.77
30	158	7	28	12.00	63.43
31	147	7	26	11.40	43.85
32	131	8	25	12.00	56.79
33	144	10	29	11.50	62.73
34	123	4	28	10.00	55.55
35	136	10	32	13.20	54.33
36	125	5	28	11.00	55.55
37	136	9	21	9.20	62.03
38	135	3	28	10.60	57.42
39	136	7	31	12.00	64.16
40	147	9	29	12.50	62.73

Appendix Table 3. Correlation Coefficients of Meiotic Characters and Seed-Set in Unselected, High and Low Populations, 1972.

Characters	Population	Cell-Variance	IV	III	II	I	No.of Chroms. in IV+II	No.of Chroms. in III+I	D.I.	Reg. M-I Cells	Reg. Tetrads	Seed-Set
Chiasma frequency	UNSELECTED	-0.271	0.029	-0.565***	0.313*	-0.736***	0.670***	-0.670***	0.632***	0.678***	0.662***	0.273
	HIGH	-0.763***	0.355*	-0.868***	0.211	-0.902***	0.825***	-0.825***	0.878***	-	0.868***	0.460**
	LOW	-0.282	-0.146	-0.594**	0.366*	-0.764***	0.677***	-0.677***	0.698***	0.694***	0.506**	-0.264
Cell-Variance for Chiasmata	UNSELECTED		-0.070	-0.087	0.070	0.097	0.014	-0.014	-0.028	-0.032	-0.085	0.044
	HIGH		-0.070	0.558***	-0.333*	0.687***	-0.592***	0.592***	-0.637***	-	-0.705***	-0.406**
	LOW		-0.020	0.553**	-0.179	0.519**	-0.561**	0.561**	-0.306	-0.406*	-0.278	0.340*
Quadrivalent (IV) frequency	UNSELECTED			-0.028	-0.863***	-0.200	0.123	-0.123	0.110	0.041	0.004	-0.123
	HIGH			-0.337	-0.810***	-0.318*	0.358**	-0.358**	0.407**	-	0.280*	0.098
	LOW			0.149	-0.939***	0.154	-0.156	0.156	-0.179	-0.147	-0.040	0.009
Trivalent (III) frequency	UNSELECTED				-0.466***	0.804***	-0.960***	0.960***	-0.896***	-0.857***	-0.544***	-0.049
	HIGH				-0.267*	0.824***	-0.914***	0.914***	-0.798***	-	-0.750***	-0.421**
	LOW				-0.475*	0.850***	-0.982***	0.982***	-0.851***	-0.805***	-0.633***	0.318

(Cont.)

(Cont.)

Characters	Population	Cell-Variance	IV	III	II	I	No.of Chroms. inIV+II	No.of Chroms. inIII+I	D.I.	Reg. M-I Cells	Reg. Tetrads	Seed-Set
Bivalent (II) frequency	UNSELECTED					-0.289	0.389**	-0.389**	0.383**	0.414**	0.296*	0.162
	HIGH					-0.239	0.211	-0.211	0.116	-	0.219	0.196
	LOW					-0.462*	0.488*	-0.488*	0.469*	0.425*	0.266	-0.119
Univalent (I) frequency	UNSELECTED						-0.909**	0.909**	-0.919**	-0.823**	-0.588**	-0.153
	HIGH						-0.828**	0.828**	-0.877**	-	-0.824**	-0.576**
	LOW						-0.935**	0.935**	-0.874**	-0.836**	-0.644**	0.284
No. of Chromosomes in IV + II	UNSELECTED							-1.000	0.931**	0.881**	0.594**	0.117
	HIGH							-1.000	0.774**	-	0.721**	0.344**
	LOW							-1.000	0.890**	0.845**	0.660**	-0.317
No. of Chromosomes in III + I	UNSELECTED								-0.931**	-0.881**	-0.594**	-0.117
	HIGH								-0.774**	-	-0.721**	-0.344**
	LOW								-0.890**	-0.845**	-0.660**	0.317

(Cont.)

(Cont.)

Characters	Population	Cell-Variance	IV	III	II	I	No.of Chroms. in IV+II	No.of Chroms. in III+I	D.I.	Reg. M-I Cells	Reg. Tetrads	Seed-Set
Disjunction Index (D.I.)	UNSELECTED									0.815***	0.554***	0.093
	HIGH									-	0.807***	0.420**
	LOW									0.782***	0.650***	-0.392*
Regular Metaphase-I Cells	UNSELECTED										0.685***	0.137
	HIGH										-	-
	LOW										0.815***	-0.173
Regular Tetrads	UNSELECTED											0.077
	HIGH											0.486**
	LOW											-0.245

n = 40 for Unselected Population
n = 40 for High Population
n = 23 for Low Population

* indicates significant at 5% level
** " " at 1% level
*** " " at 0.1% level